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THE NUCLEAR HISTORY OF SCLEROSPORA GRAMINICOLA [1]

E. S. McDONOUGH [2]

(WITH 2 FIGURES)

INTRODUCTION

Recently Hiura (**5**), Tasugi (**19**), and Evans and Harrar (**4**) showed with certainty the method of germination of the oöspores of *Sclerospora graminicola* (Sacc.) Schröt. This achievement made it possible to study critically for the first time the nuclear stages following the resting period of the oöspore. However, before this study had advanced very far it became apparent that it would also prove profitable to rework the stages in oögenesis covered by Stevens (**17**) and Ruhland (**13, 14**) some two decades ago.

REVIEW OF PREVIOUS INVESTIGATIONS

The members of the Albuginaceae which have been studied cytologically were divided by Stevens (**16**) into three groups ac-

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cording to their nuclear history preceding the origin of the oöspore. As originally described by Stevens (**15, 16**) and recently verified by Tsang (**20**), the nuclei in the oögonium as well as those in the antheridium of *Albugo Bliti* (Biv.) Kuntze and *A. Portulacae* (D. C.) Kuntze undergo two successive divisions simultaneously. The nuclei in the periplasm, however, do not undergo the second division. About 100 nuclei enter the oögonium from the antheridium and fuse in pairs with the nuclei in the oösphere. The fusion nuclei remain without dividing until the oöspore is mature.

In *Albugo Tragopogonis,* according to Stevens (**16**), a multinucleate oösphere develops, the nuclear history being similar to that of *A. Bliti.* However, the oösphere is then reduced to the uninucleate condition by disorganization of nuclei. One or more antheridial nuclei enter the oösphere and one fuses with the functional female nucleus. The fusion nucleus undergoes repeated mitoses, and the winter oöspore is consequently multinucleate.

Albugo candida (Pers.) Kuntze (**3, 16, 20, 22**) belongs to a third group in which two divisions of nuclei take place in both antheridium and oögonium and after the second division in the oöplasm all the nuclei except one are described as passing into the periplasm. Only one nucleus enters the oögonium from the antheridium. The nucleus resulting from the fusion of the two sexual nuclei undergoes several mitoses so that about 32 nuclei are found in the mature oöspore.

The members of the genus *Peronospora,* as exemplified by *Peronospora effusa* (**1, 20**), and the members of the genus *Plasmopara,* as exemplified by *Plasmopara alpina* (**12**), are similar in their nuclear history to *Albugo candida.* However, in *Peronospora* the fusion of the sexual nuclei takes place slowly and the mature oöspore contains the fusion nucleus. In *Plasmopara* the mature oöspore also contains the fusion nucleus. Another interesting feature in this genus is the small amount of periplasm formed.

While the reports of Ruhland (**13, 14**) and Stevens (**17**) on the development of *Sclerospora graminicola* are in close accord on most points, they differ with respect to important features. Stevens reports that the mitoses of all the nuclei in the young oögonium are closely simultaneous and that mitosis may proceed

until metaphase before there is any sign of differentiation into periplasm and oöplasm. When metaphase is reached the nuclei are found arranged approximately in a circle around the region that is to become the oösphere. Only one nucleus remains behind with the coenocentrum. In *Sclerospora graminicola* the coenocentrum is merely a dense mass of cytoplasm in the center of the oögonium, differentiated into two regions of different density. One of the nuclei produced by the division of the single nucleus which remains in the oöplasm wanders toward the periplasm leaving the female pronucleus near the coenocentrum. According to Ruhland, all nuclei in the young oögonium divide at the same time and after the second and following divisions, which take place near the periphery of the oögonium, a single female nucleus leaves the periplasm and enters the oösphere. This solitary nucleus remains in the oösphere and undergoes a mitosis previous to the entrance of the male nucleus. Ruhland reports one division of the nuclei in the antheridium.

Meiosis has been reported as taking place at various points in the life-cycle of members of the Peronosporales. Wager (**21, 22, 23**) did not observe meiosis during the development of the sex organs, but believed that the nuclei found in the mature oöspore of *Albugo candida* might undergo reduction in chromosome number. Stevens (**15**) in his early study on *Albugo Bliti* observed 12 chromosomes at some anaphases and six at others during the two divisions of the nuclei in the developing oögonium. Because of this he believed that meiosis might take place at this point. In his later paper (**16**) he was uncertain as to the position of meiosis in the life-cycle of the species of *Albugo* studied. However, in this report Stevens (**16**) states that the difference in character between the first and second mitosis may be due to a change in kinoplasmic content. Davis (**3**) was unable to observe meiosis in the development of the sex organs and the mature oöspore of *Albugo candida*. Krüger (**10**) reported from 14 to 16 chromosomes in the dividing nuclei of the oögonium and antheridium of *A. candida* and *Peronospora Ficariae*. All divisions taking place in these organs were described as equational in nature up to the time when fertilization took plcae. Since he observed 16 chromosomes in the nuclei formed after the first division of the fusion

nucleus, he considered that meiosis took place during the division of the fusion nucleus. Tsang (**20**) believed that the decrease in the quantity of chromatin during the first division in the oögonium had led some investigators to infer that reduction in chromosome number took place at the same time. In *Albugo candida* and *A. Tragopogonis* Tsang observed the number of chromosomes at the first anaphase of the fusion nucleus to be greater in number than at the second anaphase. The number of chromosomes at the equatorial plate of the first division was believed by Tsang to be 24. After the first two divisions the number was observed to be about 12.

METHODS AND MATERIALS

Leaves of *Setaria viridis* containing developing oögonia of *Sclerospora* were collected and fixed in August, 1932, at Ames, Iowa, and in September, 1934, near Milwaukee, Wisconsin. Some of these leaves were green and others were just turning brown. This material was fixed in Flemming's medium fluid, Bouin's picro-formal, formalin acetic alcohol, chromo-acetic solution, or a solution made up of 75 ml. of one per cent acetic acid, 20 ml. of one per cent chromic acid, and 5 ml. of 37 per cent formaldehyde. Ethyl alcohol, acetone, and butyl alcohol were tested as dehydrating agents in preparation for embedding in paraffin. Material dehydrated with butyl alcohol was the easiest to cut. Cross or longitudinal sections 5 to 10 microns thick were made and stained with Flemming's triple stain, iron haematoxylin, brazilin, or crystal-violet-iodine. The same general methods were used in the preparation of germinating oöspores for microscopical study.

Oöspore material used in studies of the nuclear aspects of oöspore germination was collected and germinated at different times during the years 1932 to 1936. The method used in germinating oöspores was essentially that described by Hiura (**6**) and was found to be the most convenient of the several tried. Petri dishes were lined with absorbent cotton which had been moistened in sterile distilled water. The oöspores to be germinated were placed on the cotton in the lower part of the Petri dish.

OBSERVATIONS

Development of the oögonium and the antheridium. Early stages in the development of the oögonium in *Sclerospora graminicola* were found to be similar to those described for *Albugo* by Istvanffi (**9**), Wager (**22**), and Stevens (**15**). The oögonium was observed to be the swollen end of a hypha, but in exceptional cases it was intercalary. The young oögonium was filled with cytoplasm and nuclei at the expense of the hypha to which it was attached. The entrance of the protoplasm was apparently rapid since a number of striae were observed in the cytoplasm and the nuclei were elongated in the direction of flow (FIG. 1: 1). At the time of entrance of the nuclei into the oögonium they were crumpled in appearance, such as has been generally described for members of the Peronosporales. Counts in serial sections of 24 young, but apparently fully expanded, oögonia indicated that from 49 to 71 nuclei had entered. Shortly after the expansion had been completed, a septum was observed to have closed the oögonium and the oögonial wall to have thickened at all points except where the antheridium was attached.

The nuclei in the oögonium were found to have increased greatly in size (FIG. 1: 2). The number of enlarged nuclei was found to range between 16 and 24 in counts based on 32 oögonia. These nuclei simultaneously pass into prophase and reach metaphase, a stage which may last, as Stevens has also reported, until differentiation into oöplasm and periplasm has begun to take place. At this point, however, no continuous membrane separates the oöplasm and periplasm, and typically all except one of the nuclei become oriented on the boundary between these regions (FIG. 1: 3). This account agrees with that of Stevens in that one nucleus is reported as remaining in the oöplasm, whereas Ruhland reports all the nuclei as migrating to the periplasm and later one nucleus entering the oöplasm.

During the expansion of the nuclei just described, there appears in the center of the oögonium a dense mass of cytoplasm which gradually increases in bulk. This body, which has been called the coenocentrum by Stevens, is, in *Sclerospora,* merely a dense mass of cytoplasm with radiating strands. One of the nuclei, which

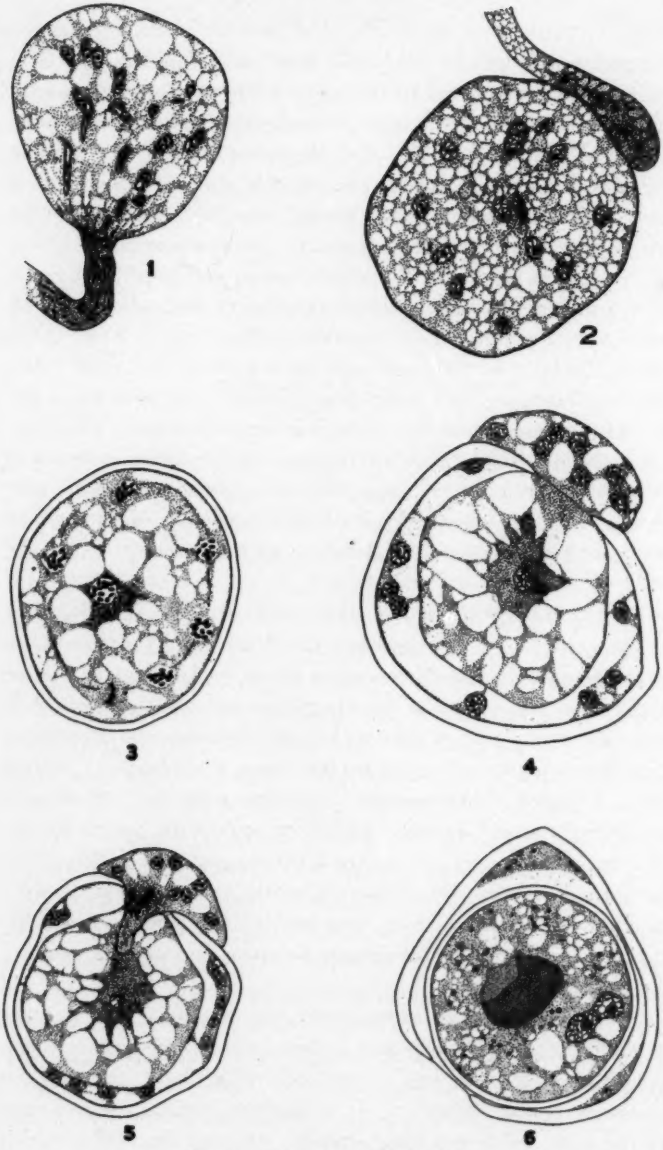

FIG. 1. *Sclerospora graminicola.*

remains close to the coenocentrum, undergoes division. One of the resulting daughter nuclei was commonly difficult to see because it may be imbedded in the coenocentrum (FIG. 1: 4).

All of the nuclei in the oögonium undergo simultaneous mitosis and the resulting nuclei enter a short resting stage, after which most, or all of them, divide again. The second divisions may not all take place at the same time. It was not unusual to find nuclei in various stages of the second mitosis. Ruhland reported the nuclei in the oögonium as undergoing several divisions, but Stevens reported only one division of the oögonial nuclei. Figure 2: 6 is interesting in this connection in that four late prophase nuclei are observed in the oöplasm. Such a figure could be explained by assuming that one nucleus has remained behind in the oöplasm and that two divisions have already taken place. Another explanation would assume that two nuclei remained in the oöplasm and have divided once and are about to undergo the second division. Further evidence that the oögonial nuclei divide at least twice is to be found in counts of nuclei made at the time of entrance of the nucleus from the antheridium. Such counts in 29 oögonia ranged from 49 to 92 nuclei. Soon after the oögonial nuclei have ceased to divide, all except one degenerate or migrate into the periplasm, the single functional female nucleus becoming imbedded in the coenocentrum.

Those nuclei which occupy the peripheral region of the oögonium have the axes of their mitotic spindles parallel to a tangent of the oögonium so that the resulting daughter nuclei do not enter the oöplasm. The nucleus remaining near the coenocentrum divides in such a way that one daughter nucleus remains in the coenocentrum.

As has been previously stated, there does not seem to be a typical simultaneous second mitosis of the nuclei since these older oögonia commonly contain nuclei in various stages of division. The second and subsequent divisions, if more than two divisions do occur, of the nuclei in the oögonium do not seem to differ in any essential way from the first mitosis. There is, however, a decided difference in the size of the chromosomes present at metaphase of the first and second divisions (FIG. 2: 4, 8). There is also a

difference in the size of the nuclei and the division figures (FIG. 2: 10, 11).

The nature of the mitoses which take place in the developing sex organs was studied in detail. The early stages in the development of the nuclei in the young oögonium were masked by their crumpled appearance. However, these nuclei soon were observed to have increased in size and were spherical in outline. The prophase shown in figure 2: 7 was similar to that of the higher plants and other fungi. As the chromatin aggregated to form the prophase chromosomes the nucleolus disappeared. In metaphase the chromosomes were clearly distinguishable. Figure 2: 8 shows 14 chromosomes present in the first mitosis in the oögonium. These chromosomes were rather small, the larger being about one micron in length and it was possible to miss the smaller chromosomes, consequently counts were difficult to make. It would seem, however, that there were not less than 14 nor more than 16 chromosomes present at this stage. The spindle was intranuclear (FIG. 2: 9) and pointed, ending in a body which stained more deeply than the surrounding cytoplasm and which might be considered to be a centrosome. This body did not, however, show in all preparations.

At anaphase the chromosomes were somewhat attenuated but the X and Y chromosomes described by Ruhland (FIG. 2: 10) were not recognized. It would seem that most or all of the chromosomes had terminal attachments. At telophase the chromosomes gradually lost their identity.

The antheridium became attached to the oögonium at a very early stage and contained at first three or four nuclei. The first mitosis preceded that of the oögonial nuclei so that by the end of the first prophase in the oögonium, the nuclei in the antheridium had already divided.

While Ruhland (**14**) reported one division of the nuclei in the antheridium, the present study seems to show that two mitoses took place. Anaphase nuclei in the antheridium at late prophase of the first oögonial division were found to be three to four in number. Eleven cases were observed in which six to eight nuclei were present in the antheridium at a slightly later stage. Mature antheridia at the time of fertilization were found to possess from 8 to 16

nuclei. These counts would seem to indicate that there were two mitoses in the antheridium.

The mitoses taking place in the antheridium were similar to those taking place in the oögonium. About 14 chromosomes were found at metaphase of both divisions (FIG. 2: 4).

Fertilization and development of the oöspore. At the point of attachment of the antheridium to the oögonium the wall of the latter remained thin. It was often impossible to distinguish between the walls of the two sex organs since they were both thin and firmly pressed together (FIG. 1: 4). Careful observation failed to demonstrate a bulging of the periplasm into the antheridium previous to the entrance of the antheridial tube into the oögonium. The projection produced in this way, the "receptive papilla" of Wager (**22**) which has been found in many members of the Peronosporales, may not be produced in *Sclerospora* or it may be very temporary in nature.

After the nuclei have ceased to divide in both of the sex organs, there may be several nuclei near the coenocentrum. However, just previous to the entrance of the conjugation tube, the oöplasm became uninucleate. Since no degenerating nuclei were observed at this stage, it is assumed that the nuclei in the oöplasm, other than the pronucleus, migrated to the periplasm. Figures suggesting this were observed and Stevens (**17**) also reported such a migration as taking place. At this stage, which is just previous to the entrance of the nucleus from the antheridium, no instance was observed where more than one nucleus was present in the oöplasm. Likewise, since "zonation," or the differentiation of the oöplasm and periplasm, takes place in *Sclerospora* rather slowly, it would be possible for nuclei to migrate from one region to another.

Shortly after zonation began, the oöplasm was displaced from the center of the oögonium toward the side to which the antheridium was attached. The oöplasm was in contact with the wall of the oögonium at a later stage (FIG. 1: 4), at which time the oösphere was surrounded by a definite membrane which Stevens called the plasmoderm.

The conjugation tube was assumed to have entered the oögonium by rupturing the thin oögonial wall. The tube apparently penetrated rapidly into the oösphere and grew some distance into the

oöplasm before it ruptured (FIG. 2; 3). Figure 1: 5 shows the penetration of the conjugation tube to approximately its greatest extent.

As shown in figure 1: 5 the conjugation tube had entered well within the oösphere before the nucleus had left the antheridium. The male pronucleus was elongated and somewhat pointed at the time of its entrance into the tube. After the nucleus had traveled the length of the conjugation tube the latter burst open allowing the nucleus as well as some cytoplasm from the antheridium to enter the oösphere. After its liberation from the tube the male pronucleus became spherical and penetrated directly into the dense mass of cytoplasm in which the female pronucleus was imbedded. Only one nucleus has been observed to enter into the oögonium from the antheridium.

The subsequent history of the conjugation tube was somewhat vague but it could be seen in the oöplasm for some time after the antheridial nucleus had entered. Apparently the tube degenerates and most of it is absorbed by the cytoplasm of the oösphere.

At first the nucleus from the antheridium was slightly smaller than the female pronucleus. However, the former soon reached the same size as the latter. In *Sclerospora* the two nuclei which ultimately fused increased in size until they were considerably larger than they were before the initial steps of fertilization took place. The sexual nuclei left the coenocentrum as that body began to disappear and the nuclei became separated some distance from each other. Nuclear fusion was delayed for a considerable time, many changes taking place in the oösphere after the entrance of the nucleus from the antheridium and previous to karyogamy.

The wall of the oöspore began to differentiate after the nucleus had entered from the antheridium. This wall was observed to be formed as a secretion of the oöplasm. During this process there might be seen directly inside the developing wall a narrow band of small granules. The wall so formed was composed of two layers, a very thin and smooth outer layer, the exospore, and a much thicker layer, the endospore. These were not easily distinguished from each other. It could thus be seen that the wall of the oöspore in *Sclerospora* was very simple when compared with that of such fungi as *Albugo candida*, in which Stevens (**16**) and

Tsang (**20**) have described the exospore as elaborate in nature and arising from the periplasm. While it is possible that the periplasm may play some part in the formation of the exospore in *Sclerospora,* this would seem unlikely since the periplasm is very much limited in extent and degenerates quickly after fertilization has taken place. Another possibility is that *Sclerospora* does not have an exospore comparable to that of *Albugo,* but that the two layers of the wall are comparable to the two layers of the endospore of *Albugo* and the thickened wall of the oögonium may be considered to be the exospore. During the formation of the wall of the oöspore, the wall of the oögonium became wrinkled and came into contact with the oöspore at several places.

As indicated in figure 1: 6 the oöspore may have developed almost to maturity before the sexual nuclei fused. The fusion took place between resting nuclei, and no instance was observed of the fusion taking place between nuclei containing chromosomes as was reported by Berlèse (**1**) for *Peronospora alsinearum* and *P. effusa.* The two nuclei came into contact with each other and their membranes dissolved away at the point of contact. The reticulum of one nucleus seemed to become continuous with that of the other. Some figures indicated that the nucleolus of the male pronucleus fused with that of the male. Such a phenomenon has also been reported for *Peronospora effusa* by Tsang (**20**). Ultimately the fusion nucleus contained but one nucleolus.

It was always possible during the course of this investigation to find binucleate oöspores which were apparently mature, judging from the condition of the central body and the oöspore walls. Since no trace of a division of the fusion nucleus was ever observed before the oöspores were placed in conditions under which they germinated, it would seem that plasmogamy might be so delayed that the mature oöspore was in some instances binucleate. However, the fusion of nuclei is normally so gradual that all functional oöspores may contain a fusion nucleus. On the other hand, among ripe oöspores, which had been soaked for six hours in a soil extract, some were found to contain stages which were identical with those of the fusion nucleus observed during the development of the oöspore. It is difficult to be certain that oöspores in which the nuclei seem to fuse after the resting period actually

germinate. However, oöspores of the same material which were fixed 12 hours later did not have nuclei which exhibited fusion stages. It would seem possible that the fusion of the sexual nuclei may in some instances be so delayed that it takes place after the resting period has been ended.

The disintegration of the antheridium started soon after fertilization and antheridia were seldom found to be present on mature oöspores. During the development of the oöspore wall the supernumerary nuclei in the oögonium began to degenerate along with the rest of the periplasm. However, it was possible to observe these nuclei after the oöspore was well developed.

Directly after the entrance of the male pronucleus, there appeared in the cytoplasm of the oöspore many small bodies which stained only slightly with any of the dyes used. As the oöspore matured these bodies became more numerous and increased in size. Eventually they coalesced in the center of the oöspore to form a central body. This body (FIG. 1: 6) which became large, completely replacing the coenocentrum, has been described by Wager (**22**) in the case of *Albugo candida* as being formed by the accumulation of oil drops. Tsang (**20**) considered this body to consist of several substances, such as metachromatin, albuminoids, phenolitic compounds, and oils. During the course of this investigation it was observed that the body in question as found in *Sclerospora graminicola* turned black with osmic acid. However, when the spores were crushed and treated with Sudan III the central body failed to give the red stain reaction characteristic of fats. In some spores containing several bodies which had evidently not as yet fused to form the large body, it was found that certain of these globules turned red with Sudan III. Even oöspores collected in the field contained globules of oil in the cytoplasm as was shown by their reaction to Sudan III and a solution of alcannin in 50 per cent alcohol. It would therefore seem that the material in question is not a typical fat.

General features of oöspore germination. Previous to the production of the germ tube, many changes took place in the oöspore. One of the most conspicuous of these was the gradual digestion of the large central body. The gross features of this phenomenon may be followed in living material but its critical details could be

seen only in stained sections. The central body seemed to have a firm, semi-solid texture. At first this body was essentially homogeneous except that it might possess a few vacuoles which contained a substance apparently of a less viscous nature. One of the first evidences of oöspore germination visible in sectioned material was the initial step in the digestion of the central body. Soon after the spores had been placed on moist cotton under the proper conditions for germination, it was observed that small pits were formed at the periphery of the central body. These pits led from the outside and extended into the mass of stored material in such a way as to produce a spongy appearance. As the germination continued these pits became larger. Eventually the pits merged and in many instances the entire body was divided into several pieces. In other cases the digestion took place more rapidly on one side than on the other. In such cases the central body presented the outline of a crescent. Ultimately, usually before the germ tube was formed, the oöspore became free of all remnants of the central body.

As has been previously shown, the oöspore is rich in oil, even in the resting condition. Tests made with Sudan III and alcannin indicated that as the central body disappeared the amount of oil in the spore increased. Germinated oöspores which had produced germ tubes were tested for oil in the same way. Many rounded oil drops were demonstrated by these tests as being present in the tubes.

Another phenomenon which may be observed in the germination process is the thinning of the wall of the oöspore. When the spore is in the resting condition this wall has been found to have an average thickness of four microns, but after the germ tube has been produced the thickness of the wall was found to average less than one micron.

Although Fréchou was reported by Prillieux (**11**) to have observed the germination of the oöspores of *Sclerospora*, the gross features of the formation of the germ tube was described with certainty by Hiura (**5**), Tasugi (**19**), Evans and Harrer (**4**), Howe (**8**), Chaudhuri (**2**), Weston and Uppal (**24**), and Takasugi and Akaishi (**18**). Chaudhuri (**2**) pictured two or more germ tubes emerging from one spore and Hiura (**7**) mentioned that

two germ tubes might leave the spore through the same opening in the oögonial wall. Oöspores have been germinated at various times during a period of more than four years, but in no case was more than one germ tube seen to emerge from an oöspore. However, it was not uncommon to find that the germ tube had branched immediately after it had emerged from the spore. Germ tubes observed under the conditions of these experiments had invariably penetrated through the thin spot which remained in the wall of the oögonium and which indicated the place at which the antheridium was attached. This thin spot was observed with ease in sections of ripe oöspores. The germ tube was hyaline in the living condition and showed a number of rounded bodies and fine granular cytoplasm.

Nuclear phenomena associated with the germination of the oöspore. During the early stages of the oöspore germination preceding the production of the germ tube, a rapid increase in the size of the nucleolus of the fusion nucleus was observed. Simultaneously with the expansion of the nucleolus, the reticulum became transformed into a mass of fine threads. At this stage (FIG. 2: 12) the average diameter of the fusion nucleus was 7.2 microns. Some indications of pairing of threads were observed, but the threads were so interwoven and so fine that it was very difficult to observe their true relationships. A noticeable feature of this stage was the position of the nucleolus. Although at first it occupied a central position in the nucleus, the nucleolus was later observed to take up a position near to, or touching, the nuclear membrane. Such figures, which were not uncommon in these early stages (FIG. 2: 12), strongly suggest the "bouquet" stage.

After the transformation of the reticulum into the thin threads of the early prophase there followed a gradual contraction of these threads. As this process advanced the visibility of the chromosomes increased until it was possible to pick out individual members of the chromosomal complement. In such stages it was possible to count at least 28 very small chromosomes. The largest chromosomes were about one micron in length; however, with proper staining and selection of filters for microscopic study the number and relationships could be determined. Such chromosomes may be observed to be in pairs. Figure 2: 13 shows the

nucleus in a prophase stage, evidently early diakinesis, exhibiting 14 bivalent chromosomes. Such figures were not uncommon, but because of the small size of the chromosomes and also because of the tendency for the nucleolus to obscure the picture, it was not always possible to make satisfactory counts. There was also a tendency for the smaller members of the complement to be obscured by the larger. Although the association of the bivalents in some instances was seen to be rather loose, in many instances, especially in the earlier stages of diakinesis, the association was close.

The later stages of diakinesis and the metaphase were found to be rather obscure, in part because these stages seemed to be of short duration and also because the large nucleolus obscured the chromosomes. It should be indicated at this point that the formation of the thickened chromosomes during the prophase of the fusion nucleus was not accompanied by a decrease in size of the nucleolus. On the other hand, this body increased in size during the prophase.

The spindle of the first division of the fusion nucleus was intranuclear. No definite centrosomes were observed and it seems likely that they are not present during the divisions of the nuclei in the oöspore. The first indication of the initiation of the anaphase was the elongation of the nucleus. This elongation was usually at right angles to the diameter of the oöspore. The spindle was not very dense and the elongation of the whole nucleus as well as the elongation of the spindle was found to be closely associated with the movement of the chromosomes to the poles. The movement of the chromosomes to the poles was not simultaneous (FIG. 2: 10). Some chromosomes were found to reach the poles before the rest and such figures presented the chromosomes so that they could be easily counted. It was not uncommon to find that one or more of the chromosomes had a decided tendency to lag behind the rest to such an extent that at late anaphase there could be seen quite clearly one or more chromosomes still close to the center of the spindle.

As has previously been stated the chromosomes were rather easy to count at early anaphase. Fourteen chromosomes were observed to go to each pole. However, it was not possible to de-

termine whether each chromosome consisted of two chromatids, nor was it possible to determine the location of the spindle attachments. Throughout the anaphase the chromosomes were observed to be in the shape of rods which at times, however, were lengthened as if they were under stress.

After the majority of the chromosomes had reached the poles of the spindle, the nucleolus began to divide. This division, because of the large size and striking character of the nucleolus, was easy to follow. Simultaneously with this division, the chromosomes began to be attached to each other by means of many fine fibers. As a result of the elongation of the nucleolus and the connecting of the chromosomes by threads, a figure was produced in which the chromosomal material formed two hollow cups in which the ends of the elongated nucleolus were imbedded (FIG. 2: 17). As the elongation of the nucleolus took place, it constricted so as to simulate a dumb-bell. It was possible, by means of the Flemming's triple stain, to stain the nucleolus red and the chromosomal material violet. Such figures presented the appearance of a large dumb-bell capped on each end with a reticulum. After the division of the nucleolus had been completed, the two nucleoli contracted somewhat. The two daughter nuclei were connected for some time by a thread which was evidently the remains of the nuclear membrane of the fusion nucleus. The two nuclei later became separated from one another by some distance (FIG. 2: 18). Subsequently the nuclei became rounded and the reticulum gradually lost its chromaticity.

The division of the two daughter nuclei was similar in its broad outline to the division of the fusion nucleus. The prophase was, however, considerably different. No pairing of chromosomes was observed and the number of chromosomes found at late prophase was 14 (FIG. 2: 15). The nucleolus divided during the division of these nuclei in a manner similar to the first division. Because of the separation of the two daughter nuclei of the fusion nucleus, it was not easy to find both of the second division figures present in the same section.

The third division taking place in the oöspore was similar in all major details to the second division. At late prophase 14 chromosomes were found to be present but this number could not be seen

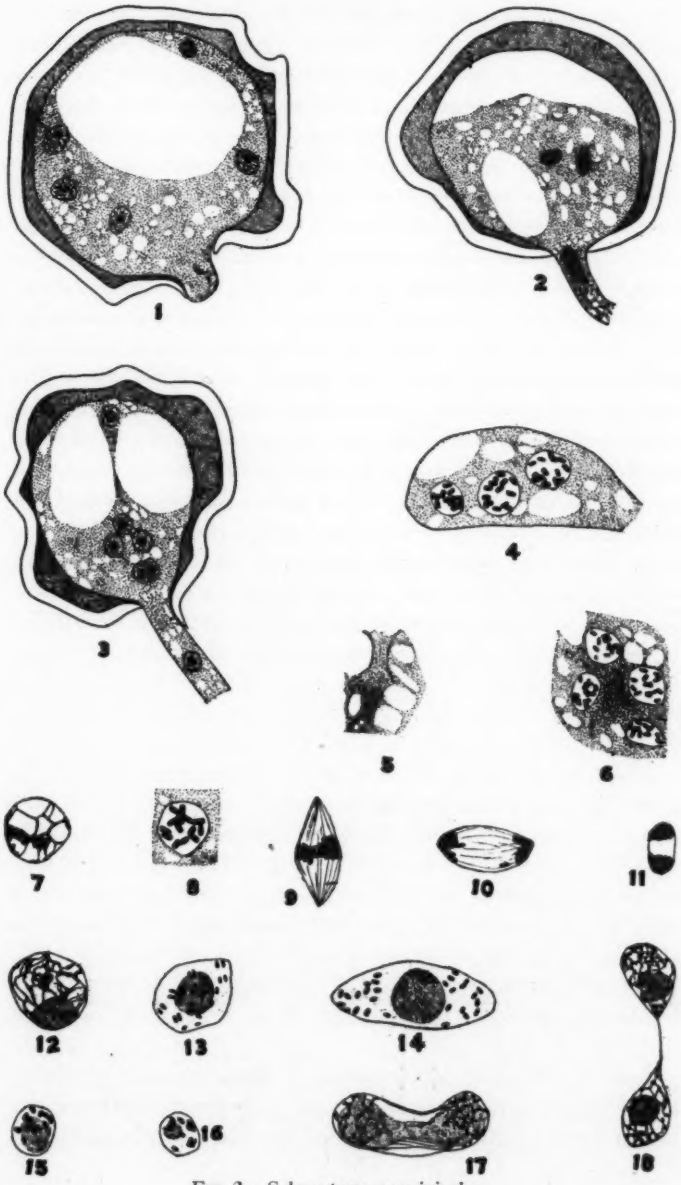

FIG. 2. *Sclerospora graminicola.*

in all nuclei because of the presence of the nucleolus (FIG. 2: 16).

It would seem that as a rule about 32 nuclei are found in the oöspore before it produces the germ tube. The later divisions taking place in the oöspore are apparently identical with the third division, but because of the decrease in size of the nuclei during this series of divisions, it was not possible to determine the character of the nucleolar division. It is possible that after the nucleolus has reached the size found in the mycelium, it disappears with each division of the nucleus in a manner similar to that described above for the nuclei in the oögonium and the antheridium.

After the nuclei had ceased to divide, or in some instances before they had ceased to divide, the wall of the oöspore, which was now thin, protruded at the spot nearest the thin place in the oögonial wall (FIG. 2: 1). After the tube had penetrated through the wall of the oögonium the nuclei began to enter the germ tube. As the nuclei entered they were seen to resemble the male pronucleus in shape (FIG. 2: 2). They were considerably beaked and elongated. In older stages of the formation of the germ tube, more than one of these beaked nuclei might be seen in readiness to enter the tube. Eventually all the nuclei might enter the tube and along with them all the cytoplasm of the oöspore. The nuclei in the germ tube were easily seen because of their conspicuous nucleoli (FIG. 2: 3).

DISCUSSION

While the studies on the development of the oögonium and the antheridium of *Sclerospora graminicola* reported here are in general agreement with those reported by Ruhland (**13, 14**) and Stevens (**17**), they differ in certain important respects. While Stevens (**17**) reported one division of the nuclei in the oögonium, some of these nuclei were observed to undergo two or more divisions, which was in accord with the work of Ruhland (**14**). Ruhland reported one division of the nuclei in the antheridium, but the present study indicates that there are normally two such divisions.

Ruhland was of the opinion that no coenocentrum was present in the oösphere, but Stevens reported that the coenocentrum resembled that of *Albugo Bliti* except that the central globule was

not observed. Stevens described the coenocentrum found in this species as a dense mass of cytoplasm. Such an accumulation was observed to be present in the oöplasm of the oöspores studied. Its extent was greater than that found by Stevens. The central globule was not observed.

Stevens and Ruhland reported the number of chromosomes present during metaphase of the division in the oögonium to be small. The number observed in the present investigation was typically 14. A certain clumping of the chromosomes was observed at times, but under proper magnification, staining, and illumination the chromosomes could be clearly seen in well preserved material.

The fusion of the nuclei is a slow process in *Sclerospora*. The evidence presented here seemed to indicate that the fusion could take place before or after the resting period. This would place *Sclerospora* in an unique position among the Albuginaceae and Peronosporaceae. However, in *Albugo Bliti, Albugo candida,* and *Peronospora effusa* Tsang (**20**) has shown that there is a tendency for not only a decrease in the number of nuclei which fuse in the oögonium, but also in the rate of fusion. In this scheme *Sclerospora* might well be considered to have carried the process still further so that fusion of the sexual nuclei takes place in the germinating oöspore.

While the divisions of the nuclei in the germinating oöspore were at times obscured by the oil drops and the disintegration of the central body, some preparations were found to be surprisingly clear. While at first the large nucleolus was confusing, it became a helpful guide to a diagnosis of the stage of the germinating oöspore. The divisions of this body were distinct and were seen to be preceded in all cases by the division and migration of the chromosomes to the poles. The gradual decrease in size of the nuclei and the nucleoli during the series of divisions taking place in the oöspore was striking. These divisions took place rapidly and the size of the nucleolus decreased out of proportion to the decrease in size of the nucleus.

While the early stages of the prophase of the fusion nucleus were not as distinct as might be desired, the later stages were sufficiently clear to indicate that the chromosomes had paired.

The anaphase stage of the dividing fusion nucleus showed the haploid number of chromosomes going to each pole. Later divisions of nuclei in the oöspore showed the haploid number of chromosomes. It is, therefore, concluded that meiosis took place during the first two divisions of the fusion nucleus.

SUMMARY

The early stages in the development of the oögonium and the antheridium of *Sclerospora graminicola* were found to be similar to those described for other members of the Peronosporales. A distortion, which was not attributed to poor fixation, was characteristic of nuclei entering the young oögonium. There were at least two divisions of most, if not all, of the nuclei in the oögonium and antheridium. The fusion of the two sexual nuclei took place very slowly, so that they had not yet fused when the oöspore had reached the resting stage. The haploid number of chromosomes was found to be present at metaphase of all mitoses in the developing sex organs.

After the nuclei in the oögonium ceased to divide, all except one were found in the periplasm. The one functional female pronucleus remained within the dark mass of cytoplasm in the center of the oöplasm. The conjugation tube penetrated into the oöplasm and one nucleus left the antheridium, passed through the conjugation tube and into the oöplasm. After the entrance of the male pronucleus, the wall of the oöspore was formed from the oöplasm and at the same time a large central body was formed. In the mature oöspore the wall of the oögonium became wrinkled and came into contact with the wall of the oöspore. The two walls were not observed to have become continuous at any point.

A few hours after the oöspores had been placed in the conditions under which they germinated the fusion nucleus divided. Subsequent divisions followed until the oöspore contained typically about 32 nuclei. At late prophase of the division of the fusion nucleus the chromosomes were seen to be associated in pairs. At anaphase the haploid number of chromosomes was found to go to each pole. Late prophase stages of dividing nuclei found during the later development of the germinating oöspore were observed

to contain the haploid number of chromosomes. The haploid number of chromosomes was found to be 14. However, the small size of the chromosomes made it difficult to be absolutely certain of the exact number. Meiosis, therefore, occurs during the first two divisions of the fusion nucleus.

During the divisions of the nuclei just described the central body was in most cases completely digested and the wall of the oögonium became thin. The germ tube arose at a point near the thin spot in the oögonial wall, at which spot the antheridium was attached to the oögonium. The tube penetrated through the wall of the oögonium at this spot. The cytoplasm and nuclei were observed to enter the germ tube. At the time of leaving the oöspore and entering into the germ tube, the nuclei were elongated and somewhat pointed.

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EXPLANATION OF FIGURES

Fig. 1. All drawings were made with the aid of a camera lucida. Magnifications are given for each drawing as reproduced. 1, × 1,350, a drawing of a young oögonium which had almost completed expansion; 2, × 1,350, a drawing of an oögonium with slightly thickened wall, to which an antheridium had become attached; 3, × 1,050, a drawing of an oöspore in which zonation and the first division of the nuclei in the oögonium have started to take place; 4, × 1,350, a drawing of an oögonium in which a mature oösphere has been produced; 5, × 1,050, a drawing of an oögonium in which the conjugation tube has entered the oösphere and the male pronucleus has started to enter the conjugation tube; 6, × 1,350, a drawing of a nearly mature oöspore in which the pronuclei were fusing.

Fig. 2. Drawings 1, 2, and 3 are reproduced at a magnification of 1,350. All other drawings are reproduced at a magnification of 2,300. 1–3, drawings of sections of germinating oöspores; 4, drawing of an antheridium in which the first division of the nuclei was taking place; 5, drawing of the

ruptured end of the conjugation tube from which the male pronucleus had entered the oöplasm; 6, a drawing of four dividing nuclei which were in the center of the oösphere; 7, a drawing of the first prophase in the oögonium; 8, a drawing of a late prophase of the first division of a nucleus in the oögonium; 9, a drawing showing the first metaphase of a nucleus in the oöplasm; 10, a drawing showing the first anaphase of a nucleus in the oöplasm; 11, a late anaphase of one of the later divisions taking place in the oögonium; 12, a fusion nucleus in prophase; 13, a fusion nucleus in diakinesis; 14, a drawing showing first anaphase of the fusion nucleus; 15, 16, drawings showing prophase nuclei of some of the later divisions which took place in the germinating oöspore; 17, an early telophase of a fusion nucleus; 18, a late telophase of a fusion nucleus.

PHOTOGRAPHS AND DESCRIPTIONS OF CUP-FUNGI—XXVI. THE GENUS DIPLOCARPA [1]

FRED J. SEAVER

(WITH 1 FIGURE)

During the summer of 1936, Mrs. Cloyd B. Stifler of Chicago brought to the writer a considerable collection of discomycetes obtained in the Pocono Mountains in Pennsylvania and in Wychwood, Wisconsin. Most of these, while excellent specimens, were well known species. However, as often happens in such collections, one turned out to be of unusual interest. The species referred to had an olive-green hymenium suggesting a *Chlorosplenium* but possessed a hairy exterior which would exclude it from that genus. After a rather strenuous search the writer succeeded in determining this as *Peziza diplocarpa* described by Currey in England in 1864. The only specimen of this species in our collection was one obtained by Massee in England. So far as can be learned this species has not been reported from North America, and apparently only twice in the world.

In addition to the olive-green hymenium and brown exterior, the species has one very conspicuous character. The paraphyses are terminated by a large fusiform tip resembling a spearhead and looks very much like a fusiform spore. It is this character which suggested the specific name *diplocarpa,* which means two-fruited. Massee claims that the spearheads are conidia and has shown them in a germinating condition. While many discomycetes have lanceolate paraphyses the writer is familiar with only one other species (*Ionomidotis irregularis*) which has this spearhead tip, and that is not at all closely related to the present form.

In 1895 Massee established a new genus *Diplocarpa* based on this species. The characters are so unusual that the writer feels

[1] This paper is preliminary to a monograph of North American Cup-fungi (inoperculates), a companion volume to North American Cup-fungi (operculates), which was published by the author and issued in December, 1928.

he was justified in doing so, and since this species is practically unknown in America it seems fitting that it should be called to the attention of mycologists. The following is the diagnosis of the genus and the one species known.

DIPLOCARPA Massee, British Fungus-Fl. **4**: 307. 1895.

Apothecia small at first closed, finally expanding and becoming shallow cup-shaped, attenuated below into a short stem-like base, densely clothed with short, septate, brown hairs giving the entire exterior a brown color; hymenium concave, olive-green; asci clavate, 8-spored; spores fusoid, containing several oil-drops and finally becoming septate; paraphyses filiform and surmounted with a fusiform conidium-like body.

DIPLOCARPA CURREYANA Massee, British Fungus-Fl. **4**: 307. 1895.

Peziza diplocarpa Currey, Trans. Linn. Soc. **24**: 153. 1864.

Lachnella diplocarpa Phill. British Discom. 232. 1893.

Apothecia gregarious or closely congested appearing sessile but actually short-stipitate at first closed then expanding and becoming shallow cup-shaped, reaching a diameter of 1–2 mm. externally dark brown, decidedly rough, tomentose, the roughening often vertically striated near the margin; hairs short septate, brown, with the tips often sharp-pointed; stem 1–1.5 mm. long, relatively thick about half as thick as long; hymenium concave, olive-green, becoming brownish with age; asci clavate, reaching a length of 70 μ and a diameter of 7 μ; spores partially biseriate ellipsoid with 2 or 3 small oil-drops, finally becoming 1 or 2-septate about $3 \times 9\,\mu$; paraphyses slender, surmounted with fusoid, septate spore-like tips which reach a length of 28–32 μ and a diameter of 6–7 μ.

On much rotted wood, Wychwood, Wisconsin.

TYPE LOCALITY: England.

DISTRIBUTION: England and Wisconsin.

ILLUSTRATIONS: Currey, Trans. Linn. Soc. **24**: *pl. 25, f. 30, 32, 33;* Phillips, British Discom. *pl. 7, figure 43.*

CONIDIA

Although Massee reports the paraphyses to be surmounted with conidia and shows them in a germinating condition, we have up to

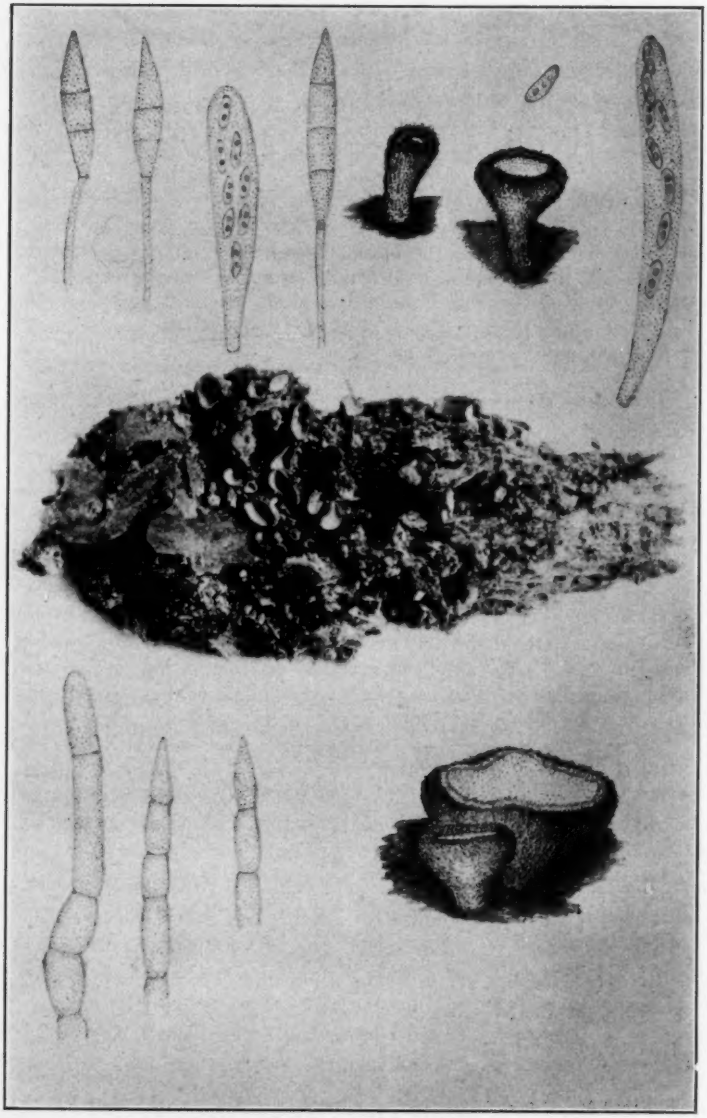

FIG. 1. *Diplocarpa Currcyana.*

date been unable to germinate those in our specimen. This does not prove anything since it is possible that they were killed in the process of drying. The collector has promised to make a search for more material, and in case it is found we will test this point further in culture.

Phillips (Brit. Discom. 338.) has called attention to the same character in *Encelia Bloxami* Phillips. He states "Fusiform, uniseptate, stylospores on slender filaments are abundantly intermixed with the asci and paraphyses, the summits rising a little above the surface of the hymenium." Drawings made from the type material by Massee show this species to be surprisingly similar to *Diplocarpa Curreyana,* except in the color of the hymenium which is brown instead of green. If the genus *Diplocarpa,* as established by Massee, is recognized Phillips' species should be included and would become **Diplocarpa Bloxami** (Phill.) comb. nov. Apparently Massee noted this similarity for a note on his drawings contains the following: "Is this the same as *Diplocarpa Curreyana?*" Phillips did not regard them as identical, and indeed they could not be, if the color of the hymenium as indicated by Phillips is correct. So far as the writer knows this species has not been found in America.

THE NEW YORK BOTANICAL GARDEN,
BRONX PARK, NEW YORK, N. Y.

EXPLANATION OF FIGURES

Center, photograph of group of plants × 4; above, drawings of two apothecia, asci and spores and three paraphyses with their conidium-like apices; below, two apothecia much enlarged and hairs from outside of an apothecium.

A NEW SPECIES OF PHLYCTOCHYTRIUM ON HYDRODICTYON RETICULATUM

J. S. KARLING

(WITH 3 FIGURES)

In connection with previous studies on *Cladochytrium replicatum* Karling another chytrid was frequently encountered which is strikingly different in several respects from any other species of *Phlyctochytrium* heretofore described. It was first observed in abundance on dead cells of *Hydrodictyon reticulatum* in battery jar cultures during the winter of 1936, and since that time has been occasionally observed on dead *Oedogonium* filaments. In no instances so far has it been found parasitizing healthy, normal green cells. Extensive attempts have been made under laboratory conditions to infect healthy filaments of *Spirogyra crassca, Cladophara* sp., *Oedogonium* sp., and *Hydrodictyon reticulatum,* but so far the results have been negative. However, when such hosts were first killed by boiling, abundant infection occurred on *H. reticulatum,* and a few thalli were found on *Oedogonium* sp. The evidence from these preliminary tests thus indicate that this chytrid is primarily a saprophyte with a rather limited range of host tissues.

The outstanding characteristic of this species is the presence of from 3 to 30 greatly elongated, hyaline comparatively stiff but flexible, radiating, branched and continuous hairs or filaments on the surface of the extramatrical zoösporangia. They begin to develop almost immediately after the zoöspores germinate, and may occasionally attain a length of 200 μ and branch several times. The intramatrical sub-sporangial swelling or apophysis varies greatly in size and shape, and on its base is oriented an extensive rhizoidal system. The extramatrical resting spores are hyaline, smooth, thick-walled and oval to spherical in shape. These characteristics together with its method of development places this species well within the limits of the genus *Phlycto-*

chytrium, as established by Schröter (**1897**), and at present there appears to be no question as to its identity and synonomy with other species which are characterized by setae or occur on *Hydrodictyon reticulatum.* This alga appears to be comparatively resistant to attack by chytrids, and up to the present time only two species have been reported on it, *Phlyctochytrium Hydrodictyi* by Braun (**1855**) and *Hyphochytrium Hydrodictyi* by Valkanov (**1929**). The former has been recorded three times since Braun discovered it, and the latter only once. The present species is quite different from either of these two, and there can be no doubt as to its distinction. In 1931 Valkanov described a chaetophorous species, *Rhizophidium* v. *Mindeni,* which Sparrow (**1933**) thinks is synonymous with Scherffel's (**1925**) *Chytridium chaetophilum,* on the oögonia of *Oedogonium* whose zoösporangia bear a superficial resemblance to those of our species, but the intramatrical system is quite different. It appears thus that we are dealing with a new and undescribed member of *Phylctochytrium,* and with the view of emphasizing its outstanding characteristic I am proposing the specific name *chaetiferum* for this species.

Phlyctochytrium chaetiferum sp. nov.

Zoösporangia gregarious, sessile extramatrical, predominantly pyriform and oval in shape, $12 \times 18\,\mu$ to $30 \times 45\,\mu$ in diameter; possessing from 3 to 30 elongated, flexible, branching hairs which are approximately $2\,\mu$ in cross section at their point of insertion and sometimes attain a length of $200\,\mu$. Intramatrical apophysis or sub-sporangium spherical (8–11 μ) irregular, elongated or spindle-shaped with one to several rhizoids oriented on its base or sides. Zoöspores spherical, 2.5 to $4\,\mu$ in diameter with a single posteriorly attached cilium and a highly refractive globule in the center. Resting spores extramatrical, hyaline, smooth, thick-walled, oval and almost spherical, 9×10 to $14 \times 17\,\mu$ in diameter. Apparently saprophytic on dead cells of *Hydrodictyon reticulatum* and *Oedogonium* sp., New York City.

GERMINATION OF THE ZOÖSPORES AND DEVELOPMENT OF THE INTRAMATRICAL THALLUS

The zoöspores of *P. chaetiferum* are spherical, hyaline, 2.5 to $4\,\mu$ in diameter with a conspicuous clear refractive globule in the

center and a single posteriorly attached cilium which is approximately four times the diameter of the spore in length. Their general appearance and habit of swimming strikingly similar to that of other species of this genus, and nothing significantly different has so far been observed. They may frequently become amoeboid under conditions adverse to free swimming and drag their cilium behind, but when liberated they round up and dash off again. After a motile period which varies from 20 to 80 minutes they gradually come to rest, lose their cilium, and either degenerate or germinate. Under the laboratory conditions of this

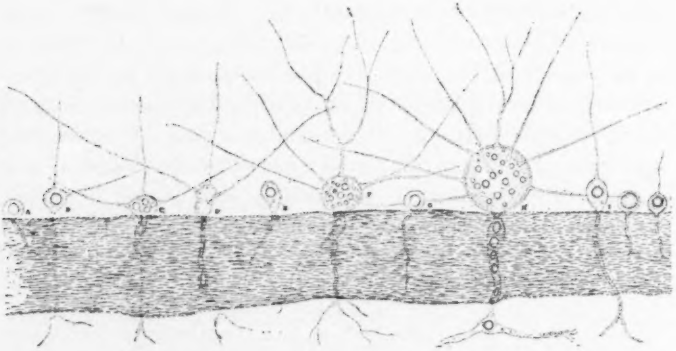

FIG. 1. Germination of the zoöspores and early developmental stages of *Phlyctochytrium chaetiferum* on a *Hydrodictyon* cell.

study a fairly high percentage of them have degenerated in the surrounding water. The remaining ones which come to rest on the host cell soon put forth a clearly visible germ tube which penetrates the cell wall as is shown in figures 1*A*, 1*E*, 1*G* and 1*J*. Figure 1 is a drawing of a portion of a *Hydrodictyon* cell on which were a large number of thalli in various stages of development and gives rather an exceptional view of this chytrid's relation to the host. The germ tube grows through the wall, and shortly after entering the lumen of the cell begins to branch, figure 1*B*. In some instances branching may occur within the host cell wall, as is suggested by figure 1*F*. These branches are the rudiments of the rhizoidal system, and as is shown in figures 1*B* and 1*D* the

chytrid very early lays down the anlage of an absorbing system within the host cell.

Very shortly after this branching has occurred the germ tube begins to swell and enlarge in a localized region just above the point of origin of the rhizoidal branches. This swelling increases in size and eventually becomes the intramatrical apophysis. The rhizoids become more and more oriented, particularly in the case of the spherical sub-sporangia, on its base, and one often gets the impression that the former was the first of the two to be developed. The apophysis usually lies immediately within the host cell, but

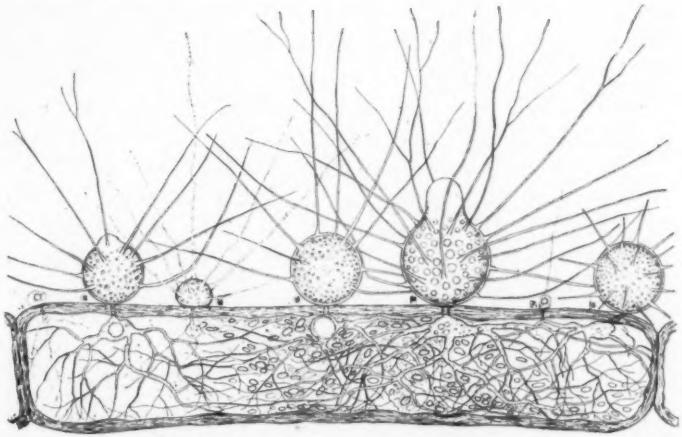

FIG. 2. *Phlyctochytrium chaetiferum* on a short *Oedogonium* cell.

in the case of old hibernating, winter nets it may often develop within the layers of the wall, as is shown in figures 1*F* and 1*H*. The mature apophysis varies considerably in size and shape, and it is accordingly difficult to give accurate and representative measurements. It may be almost spherical, 8 to 11 μ, figures 2*D*, 3*E* and 3*F*; irregular, figures 2*B*, 2*E*, and 2*G*; or reduced to an elongated and cylindrical tube, figures 3*C*, 3*D* and 3*I*; or somewhat spindle-shaped, figures 3*G* and 3*H*. In the majority of thalli so far observed the rhizoidal branches have been fairly closely oriented on the base of the apophysis, but this is by no means the general rule. In the case of the irregular ones shown in figures 2*B*, 2*E*,

and 2*G* they may originate at various places on the surface. The rhizoidal system is well developed and extensive in distribution as is shown in figure 2. At their point of insertion on the apophysis the branches may be as much as 5 μ in diameter, but as they branch and ramify among the starch grains in the *Hydrodictyon* cells they diminish rapidly in thickness and eventually run out to fine points.

DEVELOPMENT OF THE ZOÖSPORANGIUM

Simultaneous with the branching of the penetration tube and the establishment of the anlage of the rhizoids and apophysis the hyaline setae appear on the surface of the extramatrical zoöspore. In a few cases they have begun to develop before the germ tube has completely penetrated the host cell wall, figure 1*K*, while in others they have been considerably delayed. Usually a single or two protoplasmic hairs appears at the apex, figures 1*I*, 1*K*, 2*F* and 1*B*, but as the zoöspore begins to enlarge in size others are formed. They begin as minute projections or papillae on the surface, and then elongate rapidly without much additional increase in diameter, so that at maturity they are never more than 1.7 to 2.5 μ in diameter at the point of insertion. They may branch several times, as is shown in figures 1*F* and 1*H*, and when fully developed may extend for a distance of 200 μ in exceptional cases. As many as thirty hairs have been counted on a single zoösporangium. They are hyaline in color, continuous with the sporangia, filled with finely granular greyish protoplasm, and are comparatively stiff but readily flexible. This has been particularly evident in cultures of *Hydrodictyon* cells when rotifers were unusually abundant. Quite frequently the setae have been badly bent by such animals while feeding and crawling about, but when the pressure was released they sprang back into the original position. It is not uncommon, however, to find the bent and broken on mature and empty zoösporangia.

During germination of the zoöspore the refractive globule may remain in the spore body and increase in size or fragment into a number of smaller bodies. At least this often appears to be the case as is shown in figure 1*C*, although the successive stages have not been observed. Quite frequently similar globules may appear

in the penetration tube and incipient apophysis, figures 1*D*, 1*F* and 1*H*, and at maturity a very large refringent body of the same type may be found in the latter as is illustrated in figures 2*B* and 2*D*. As the zoöspore enlarges it may become somewhat flattened and dome-shaped on the surface of the host cell, figures 1*C* and 2*C*, but occasional double and constricted ones such as is shown in figure 1*D* may occur. At this stage the incipient zoösporangium usually appears well vacuolated, suggesting that growth in the early developmental stages is largely the result of imbibition of water. Figure 1*F* shows a more advanced zoösporangium which contains a number of refringent bodies of various sizes suspended in a rather dense and finely granular hyaloplasm. A later stage of development is shown in figure 1*H* in which the zoösporangium has almost attained mature size. The refractive globules are less in number and larger, which seems to be due to a coalescence of the smaller ones as the protoplasm matures. The setae extend in all directions except towards the host cell, while the intramatrical apophysis is reduced to scarcely more than an elongated tube. Following this stage a comparatively hyaline and clear region begins to protrude at the apex of the zoösporangium, figure 2*B*, which elongates considerably as is shown in figure 2*E* and eventually becomes the dehiscence papilla. As a result of this growth the zoösporangia usually becomes somewhat pyriform in shape, but it is not uncommon to find almost spherical ones also. The sporangium shown in figure 2*E* has reached mature size and is soon ready to undergo cleavage. The round refringent globules are approximately equal in size and except in the dehiscence papilla more or less evenly distributed throughout. Cleavage occurs in such a fashion that a single globule is included in each segment. Although the successive stages have not been followed it is without doubt progressive and predominantly centripetal in direction.

The mature zoösporangia of *P. chaetiferum* vary considerably in size and shape. The majority are somewhat pyriform, but almost spherical ones are not uncommon. The former vary from $12 \times 18\,\mu$ to $30 \times 45\,\mu$, while the latter range from 15 to $47\,\mu$ in diameter.

Shortly after cleavage has been completed the dehiscence papilla opens and the zoöspores begin to escape. Only a limited

number of dehiscing zoösporangia have been observed and as yet it is difficult to draw definite conclusions as to the type method of exit and immediate behavior of the zoöspores. In a few instances the fully delimited zoöspores have escaped in a mass surrounded by a faint film or membrane and remained quiescent at the mouth of the zoösporangium, figure 3*A*, for a few minutes. Then as this film disintegrated they began to slowly move apart and swim

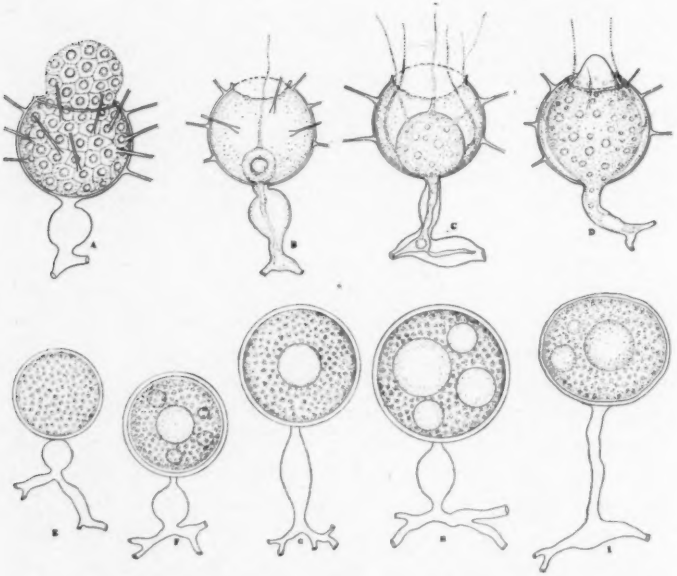

FIG. 3. Exit of the zoöspores, germination and development *in situ*, and the resting spores of *Phlyctochytrium chaetiferum*.

away. Whether the film is a surface tension membrane or a morphological one is difficult to determine in living material. Its refractive index is quite similar to that of the zoöspores, and may be readily overlooked. In other zoösporangia the zoöspores have escaped independently one by one without the presence of the membrane. It is not improbable in such cases that the membrane or film may break in the region of the dehiscence papilla as the zoösporangium opens, and the zoöspores are thus enabled to

emerge free and independent. The pressure of the cover glass and other mechanical factors may possibly influence the manner of exit also.

Quite often a number of zoöspores may fail to escape entirely, and frequently germination *in situ* occurs. The germ tubes may grow through the wall of the zoösporangium or down into the apophysis and rhizoids as is shown in figure 3*B*. Such thalli may continue to develop, and in two instances have been found to attain maturity. Figures 3*C* and 3*D* show such developmental stages. From such appearances one may readily get the impression that the zoösporangia of *P. chaetiferum* undergo proliferation, and quite recently Sparrow (**1936**) has interpreted similar thalli as such in *Rhizophidium simplex*. That this is not the case in the present species becomes evident when the successive developmental stages are followed.

RESTING SPORES

The resting spores of *P. chaetiferum* are hyaline, smooth, oval to almost spherical in shape, with walls approximately 2 μ thick and usually one to several large refractive globules lying in the center as is shown in figures 3*E* and 3*I*. The spherical ones may vary from 10 to 17 μ, and the oval ones $9 \times 10\,\mu$ to $14 \times 17\,\mu$ in diameter. They occur on the outside of the host cell in the same fashion as the zoösporangia, and usually develop in a culture after the latter have begun to disappear. So far no sexuality has been observed in their formation, nor have any germination stages been found.

SUMMARY

1. *Phlyctochytrium chaetiferum* has been found on dead cells and filaments of *Hydrodictyon reticulatum* and *Oedogonium* sp. which had been growing in battery jars in the laboratory. It is characterized by oval and somewhat pyriform extramatrical zoösporangia on which occur from 3 to 30 elongated, hyaline, continuous, branched, comparatively stiff but flexible hairs which may occasionally attain a length of 200 μ. The intramatrical portion of the thallus consist of a globular, spindle-shaped, elongated, or irregular apophysis and a fairly extensive rhizoidal system oriented on its base or sides.

2. The zoöspores are hyaline, spherical, 2.5 to 4 μ in diameter, with a large clear refractive globule in the center and a single posteriorly attached cilium which is approximately four times the diameter of the spore in length.

3. The resting spores are extramatrical, hyaline, oval to spherical in shape, with smooth thick walls and one or more large refringent globules in the center. So far no gametic fusion has been observed in relation to their development, nor have any germination stages been found.

BOTANY DEPARTMENT,
COLUMBIA UNIVERSITY

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CROZIER FORMATION IN THE GYMNOASCACEAE: A PRELIMINARY NOTE[1]

EDWARD D. DE LAMATER

(WITH 33 FIGURES)

INTRODUCTION

In April, 1935, some pellets of hair and feathers were found about a buzzard roost near Lock Raven, Maryland. These balls of material, apparently regurgitated by the buzzards, were placed in a moist chamber in the laboratory. About two weeks later a fluffy, yellow, mycelial growth was observed. The fruiting fungus was tentatively identified by Dr. C. L. Shear of the Bureau of Plant Industry as being *Arachniotus aureus* (Eidam) Schröter.

When cultures of a fungus regarded as the same species by Nannizzi were obtained from Baarn, Holland, and were compared with the organism from the buzzard castings, it became apparent that the two were quite distinct. A study of the original literature, as well as Saccardo's treatis of members of the family Gymnoascaceae, in the Sylloge Fungorum, shows clearly that the diagnoses are too inadequate to permit certain identification with them of fungi which may be found elsewhere. It seems certain that not only Nannizzi's species and the form from buzzard pellets, but also a dozen other species of the family now under cultivation in this laboratory, are actual bonafide members of the Gymnoascaceae. It is the writer's plan to make comparative studies of these during the next few years.

The writer is indebted to Dr. D. S. Johnson for facilities in carrying out his work, to Dr. J. N. Couch for his helpful suggestions, to Dr. C. L. Shear for his continued interest, and to Dr. D. H. Linder for transmission of valuable information.

[1] It would seem that the species discussed here is undoubtedly new, but no attempt will be made to name it until a comparative review of the group is presented.

HISTORY

Frederick Currey (**1854**), as I have learned from Dr. D. H. Linder, apparently described the first fungus belonging to the Gymnoascaceae. This fact seems to have been overlooked by most later workers.

Baranetsky (**1872**) described *Gymnoascus Reessii* in considerable detail, and founded the genus upon which the family is based. Eidam (**1883** (*a*), **1883** (*c*), **1886**) added appreciably to the numbers of known types in the group, as well as a good share of the total present day knowledge of their structure and sexuality. Brefeld (**1891**), Zukal (**1890**), Van Tieghem (**1877**), Matruchot and Dassonville (**1899, 1901**), Dangeard (**1903–1907**), and others have also contributed, but even so the available information is scant. Dale (**1903**) summarized the literature and studied morphologically and cytologically three members of the family, one very incompletely. Due partly to the small size of the fungi studied, and partly perhaps to the inadequate optical equipment available to her, Dale either failed to observe certain structures or failed to interpret them correctly. Her account, inadequate as it is, is unquestionably the most complete yet published for any of the organisms of this family. It is because of the possible reinterpretation of certain of Dale's figures from evidence obtained from the study of the development of this new (and interesting) form of *Arachniotus* here reported that this brief preliminary account is presented.

METHODS

Van Tieghem cells were used in the study of single spore inoculations, of spore germination, and of early hyphal stages. A Spencer micromanipulator was used for spore isolation. Observation for general characters, as well as for early sexual stages, were made on whole mounts of living material, and on aceto-carmine preparations (Emmons, **1935**). The latter were made permanent by Buck's method (**1935**).

Most of the detailed study was done with paraffin sections of 4 μ to 15 μ in thickness. Schaudinn's and Gilson's fluids were the most satisfactory fixatives. Iron alum haematoxylin proved to be the most satisfactory stain. Sections stained with Haema-

toxylon (Haedenhein's) were destained in a saturated solution of picric acid in water, followed by dilute ammonium hydroxide to clear the picric acid and differentiate more clearly. (Ref. also Summers, **1935**.) Other strains, such as Gram's fungus stain and Brazilin, have not been as useful. The Feulgen reaction has not as yet given consistent or satisfactory results, in this work.

THE ORGANISM

1. *Spore germination:* Spore germination occurs between 24 and 36 hours after the sowing of the spores on agar, the time depending upon the temperature. At first the spore swells to three or four times its original volume (FIG. 1 *a, b, c, d*); then, apparently anywhere on the spore surface, but usually at the narrower ends, a rupture occurs in the outer, heavy spore coat (FIG. 1 *c, d*) through which a small papilla or germ tube begins to protrude (FIG. 1 *d*). During succeeding hours the germ tube elongates (FIG. 1 *e, f*) and soon may or may not branch. Sometimes also two germ tubes may push out of the same spore at different places (FIG. 1 *e*); the second most commonly occurring on the side opposite the first. The spore coats have been observed to persist three or four days after germination clinging to the hypha as ragged, dark masses. Septations do not appear in the young hyphae until several hours after spore germination as a rule. The nuclear stages here have not as yet been followed.

2. *The vegetative mycelium:* The hyphae during vegetative growth vary considerably in thickness, both in aerial and subaerial mycelia. Ordinarily in actively growing, young cultures the hyphae are densely filled with cytoplasm, but in older stages in both layers of growth they become variously vacuolated. Hyphal fusions are of common occurrence in both kinds of mycelia. Figure 33 is a photomicrograph of such a fusion between two aerial hyphae. Figure 2 shows variations in hyphal thickness and also a characteristic swollen cell. Asexual spores are apparently formed only under very wet conditions, such as an agar plate flooded with water or in types of liquid media such as, potato-juice and sucrose. This is quite different from Nannizzi's fungus which forms abundant conidia under ordinary conditions.

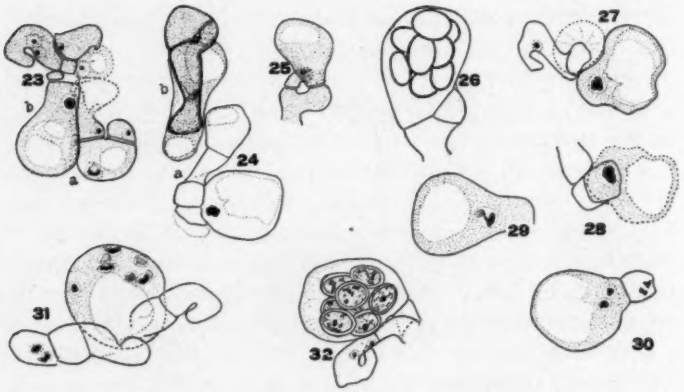
FIGS. 1–22.

The mycelia of *A. aureus* have been grown on potato-sucrose agar and on Sabouraud's glucose-peptone agar, as well as on several other media. On the first two media mentioned, the growth is much the same, except that on Sabouraud's radial furrows appear in the mycelium. At first, on both media, the growth is imbedded in the surface of the agar, but soon, the time depending on the wetness of the culture, a fluffy aerial mycelium appears. Vegetative cultures in which the aerial mycelium has arisen are pure white. The sexual phase is produced on both media, the time of initiation depending here apparently upon such factors as the light and water relations of the fungus. Such physiological factors need further investigation.

At the advent of the sexual phase a faint yellowish tint appears in the aerial mycelium, the occurrence of which seems to be a definite sign that the sexual phase has begun. Nannizzi notes the fact that sexuality is always accompanied by a similar color change in the European type of *A. aureus*. Furthermore it appears that the intensity of the color, which gradually deepens as maturity is reached, is a direct indication of the stage of sexual development reached by the fungus, and that light is evidently one of the primary factors affecting the initiation of the sexual organs; hence it may be found that here is a definite arbitrary index for a physiological study of the influence of light on sexuality. Examination of preparations made from any culture at a given time after the initiation of the color phase, shows that the sexual development of all male and female branches is, within limits, in about the same stage. Cultures grown in partial or total darkness fruit most readily. Wet cultures, as noted by Dale, fruit less rapidly.

3. *Origin of ascocarps:* The male and female branches may arise apparently from either the same or separate hyphae (FIG. 2, 3, 4, 5, 8). They arise much as described by Barenetsky for *Gymnoascus Reessii,* Eidam for *G. candidus,* Dale for both species. Nothing simulating what Eidam and Brefeld described for *G. Reessii* has ever been observed in this *Arachniotus aureus.* Eidam considered that a sexual branch coiled either about the parent hypha or about a neighboring hypha, and then gave rise to the sexual products, whereas Brefeld claimed a single sexual branch arose and subsequently gave rise to ascogenous hyphae and

asci without coiling either about another sexual branch or about the parent filament. The sexual branches appear as two small papillae or swollen knobs which lie closely, side by side, and are identical in appearance. These elongate, swell, and begin a very slight mutual coiling, one about the other (FIG. 4, 5) which never progresses very far. At this stage a wall separates each from its parent hypha and each branch is found to be uninucleate (FIG. 5). The fusion of the male and female cells may occur soon after this stage is reached or not until a much later time. Dale notes vari-



FIGS. 23–32.

ability in time of fusion and also variation in the dependent shape of the ascocarp. The writer regards the occurrence of fusion as a fairly well established fact, not only in this form but in *G. Reessii* and *G. candidus* as shown by Dale.

During further development the male branch elongates to a rather straight, club-like central cell, while the female branch (so designated because it gives rise to the ascogenous hyphae) continues to wind about the male in a coil of varying tightness. When fusion occurs early in the development, the male cell elongates beyond the point of fusion while the female also grows beyond this point coiling as it grows. If fusion occurs later, it is usually at or near the tips of the enlarged male and of the respectively coiled female cell. During these stages the number

of nuclei in both sexual branches increases. No evidence for the coiling about both sexual branches of a process derived from the female cell, as described by Dale, has ever been observed. About the time the branches have reached the stage shown in Figure 11, the coiling female begins to be cut up into cells which are usually very nearly isodiametric (FIG. 13, 15, 16). These are always binucleate (FIG. 16). It is believed that one of the two nuclei in each cell is derived from the original male and the other from the original female nucleus; although it will be difficult to establish this beyond doubt.

4. *Crozier formation:* Soon after the formation of the binucleate cells of the female branch, a process which is the fundament of a crozier begins to bulge out from each of them (FIG. 17). The two nuclei migrate into the characteristic hook and there undergo a simultaneous division (FIG. 19), followed by cell walls by which two non-sister nuclei are isolated in the bend of the hook, while one of the other pair is relegated to the base (pedicel) of the crozier and the other to the tip cell (FIG. 20, 21, 22).

5. *The ascus:* The binucleate cell at the bend of the crozier (cell *a*, FIG. 23) then begins to enlarge to form the ascus. At the initiation of this stage a vacuole appears, and, as the cell enlarges, the vacuole enlarges coincidently. A large vacuole is characteristic of the developing ascus, but it finally disappears about the time of spore formation.

Typically in crozier-forming Ascomycetes the uninucleate tip cell of the crozier is capable of fusion with the uninucleate basal (or pedicel) cell, thus reestablishing the binucleate condition (FIG. 24 *b*, 25, 26). When this occurs the newly established binucleate cell may then grow out to form a new crozier, and the above described process of ascus formation is repeated. Thus many asci may be formed from the relatively few original cells of the coiled female branch. Such a process, admirably diagrammed by Claussen, is believed to explain Dale's observations that the ascogenous hyphae in *G. Reessii* and *G. candidus* branch repeatedly forming dense clumps, each clump arising from one of the isodiametric cells of the coiled ascogonium. Dale evidently did not work out these later stages. No sterile cells have ever been observed to arise from the base of the ascocarp, consequently clusters of asci each de-

rived from a single sex act are merely nestled at random among the aerial hyphae with no other special protective sterile cells about them.

Dale's figures of early stages give fairly conclusive evidence that croziers are formed. Her figure 17 shows the segmentation of the female branch very clearly for *G. Reessii.* Figure 18 shows structures which are very like the croziers here drawn and described by the writer. In her consideration of *G. candidus* her figures 46 *a*, 46 *b*, 47 *a*, and 48 *b* show very clearly structures which, when compared with those described here (FIG. 17, 18, 19, 20, 21, 22), may well be considered to have the same significance. Her figure 48 *b* even suggests the formation of walls in the young crozier. The figures and descriptions given by Dangeard (**1903–1907**), Eidam (**1883** (*a*), (*c*), **1886**), Van Tieghem (**1877**), and others are not sufficiently detailed to draw any possible homologies from. It is of particular interest to find crosiers in this species since it represents one of the lowest (possibly the lowest) of the Ascomycetes in which crozier formation has been reported.

Nuclear fusion has been demonstrated to occur in the young ascus of this *Arachniotus.* Figures 23 *a*, 24 *a*, 27, 28 show the establishment of this condition. The time of nuclear fusion may vary considerably, if the size and shape of the ascus can be considered as an indication of its maturity. Characteristically, by three subsequent divisions of this fusion nucleus eight spore nuclei are formed, about each of which a spore is delimited. The mode of spore delimitation seems to resemble that described by Harper (**1900**) in *Pyronema.*

The length of the pedicels of the asci, the presence or absence of which has been regarded as an important taxonomic character in these forms, is here so variable as to be of questionable taxonomic value.

TYPE OF SEXUALITY

In July and August, 1935, an attempt was made to determine whether this organism is hermaphrodite and sterile, as in *Pleurage anserina* and *Ascobolus magnificus,* or whether it might prove to be hermaphrodite and fertile as in *Pyronema confluens.* Curiosity as to the sexuality was aroused by the observations of hyphal fu-

sions between aerial hyphae bearing male and female branches (FIG. 6 *a*), and the early, preliminary observations which suggested that the male and female branches invariably arise from separate hyphae.

Some fifty single ascospore cultures each produced perithecia in which normal eight spored asci were to be found, thus indicating that the organism is either hermaphrodite and fertile or parthenogenetic. It may be said that cytological results so far

FIG. 33. Photomicrograph of hyphal fusion between two aerial hyphae. Living material.

indicate, but do not prove, that this fungus is not parthenogenetic, but is a true case of hermaphroditism and self-fertility. The original uninucleate condition of each of the sex branches, the subsequent cellular fusion of the male and female branches, the binucleate condition of the cells of the ascogonium, and the activity of these nuclei in the subsequent stages of crozier formation seem to the writer to be very suggestive evidence for the above conclusion. The binucleate condition is very obvious in all material properly stained to show it. From the evidence so far available on this *Arachniotus aureus* from buzzard castings it would appear that the nuclear cycle and sexuality is like that of *Pyronema confluens* (Claussen, **1912**).

DISCUSSION

The significance of the finding of croziers and simultaneous nuclear divisions in the formation of asci, become evident upon comparison with *Pyronema confluens* and other such supposedly higher ascomycetes, as well as on comparison with forms lower in the phylogenetic system, such as *Eremascus albus* (Eidam, **1883** (*b*)), in which the single ascus is derived directly from the ascogone itself. It is not surprising to find a true sexual process here, since such a process with a multitude of variations is known to occur in the yeasts, but it certainly is both surprising and of prime significance in the building of a phylogenetic system, to find a mechanism which has usually been considered as characteristic for "higher" ascomycetes. Croziers are known to occur occasionally in the fungi comprising the Aspergillaceae, as has been shown by Emmons (**1935**) for *Byssochlamys fulva* Oliver and Smith. This was the only case among thirteen species studied. Croziers are also formed in *P. avellaneum*, as found by the writer (unpublished). In addition Emmons found three striking cases where the perithecia were distinctly gymnoascus-like, and others which were but little more complex. It would seem that this *Arachniotus* is probably the most primitive ascomycete in which croziers have been described.

SUMMARY

1. A new fungus regarded provisionally as *Arachniotus aureus* (Eidam) Schröter, was found on pellets apparently regurgitated by buzzards.

2. Upon comparison with Nannizzi's species by the same name, there was found to be no concurrence between the characters of the two fungi.

3. A preliminary study of the development showed:

(*a*) a true sexual process to occur, and

(*b*) crosier formation accompanied by conjugate nuclear division in the formation of asci.

The stages are compared with figures drawn but not interpreted by Dale (**1903**).

4. Study of single ascospore cultures along with the cytological findings now available show that this species is probably monoecious and self-fertile.

5. The significance and importance of the occurrence of croziers and monoeciousness in this and related organisms is emphasized.

6. The naming of this apparently new fungus is being left until a more comprehensive study of the family is presented.

DEPT. OF BOTANY,
JOHNS HOPKINS UNIV.

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EXPLANATION OF FIGURES

All drawings were made with the aid of a camera lucida.

Fig. 1. Germinating spores: *a, b, c, d*—early stages showing swelling of spores, rupture of spore coat, and sprouting of germ tube; *e, f, g, h*—slightly later stages showing branching and origin of two germ tubes from one spore. × 385, all from living material. Fig. 2. Aerial hyphae showing variation in size, characteristic swollen cell, origin of male and female branches from one hypha. × 1335, glycerine mount. Fig. 3. Young ascogonium and antheridium arising from different hyphae; living material. Fig. 4. Slightly older ascogonium and antheridium; living material. Fig. 5. Ascogonium and antheridium in uninucleate condition, with basal wall formed. × 1335. Fig. 6. Ascogonium and antheridium from hyphae between which there is a hyphal fusion, *a*, but a few micra from the sexual branches; living material. Fig. 7. Later stage of ascocarp; chromatic masses in antheridium. Male cell, club shaped; female, coiling. × 1335. Fig. 8. Stage as in 7. Male and female from same hypha. Acetocarmine mount. × 1335. Fig. 9. Stage as in 7. Peculiar trichogyne like process, *t*. Living material. Fig. 10. Male and female branches showing cellular fusion. × 1335. Fig. 11. Male and female branches prior to segmentation of ascogonium. × 1335. Fig. 12 and 13. Cross-sections of male and female branches at point of cellular fusion. × 1335. Fig. 14. Longitudinal optical section showing division figures in ascogonium. × 1335. Fig. 15 and 16. Optical cross-section of ascocarps showing segmentation of female. Fig. 16 shows binucleate condition of female cells. × 1335. Fig. 17. Optical cross-section; early stages in crozier formation. × 1335. Fig. 18. Four croziers in slightly older stage. × 1335. Fig. 19. Simultaneous division of two nuclei in crozier. × 1335. Fig. 20. Crozier in which two non-sister nuclei segregated to bend, one in tip and one in base of crozier, prior to wall formation. × 1335. Fig. 21. Production of young binucleate ascus by formation of cross-walls. × 1335. Fig. 22. Longitudinal section through ascocarp showing crozier as in 21. × 1335. Fig. 23. Enlargement of young ascus, *a*, in which characteristic vacuole is forming. Nuclei possibly fusing. *b*, Older stage of ascus development. Large fusion nucleus present. × 1335. Fig. 24–26. Showing fusion of tip cell of crozier with basal cell. Fig. 26, late stage. × 1335. Fig. 27 and 28. Older uninucleate asci. Nucleus in Fig. 27 probably fusing. × 1335. Fig. 29. Possible first division stage in ascus. × 1335. Fig. 30. Binucleate condition in older ascus. Note binucleate condition of Basal cell. × 1335. Fig. 31. Eight-nucleate condition of ascus prior to spore formation. Nuclei at different levels. × 1335. Fig. 32. Aspect of ascus and spores stained with iron alum haematoxylin. × 1335.

UNDESCRIBED SPECIES OF CERCOSPORELLA AND CERCOSPORA ON CERTAIN GRASSES IN OREGON AND WASHINGTON[1]

RODERICK SPRAGUE[2]

(WITH 3 FIGURES)

During investigations on the host range of *Cercosporella herpotrichoides* Fron on cereals and other grasses (**5**), three apparently undescribed species of *Cercospora*-like fungi were found, one each on *Holcus lanatus* L. and *Bromus rigidus* Roth in the field, and a third on *Melica subulata* (Griseb.) Scribn. on an herbarium specimen of this grass collected by the late D. C. Ingram. The writer uses the term "*Cercospora*-like" on account of the partially accepted practice of including *Cercosporella* and *Cercosporina* in the genus *Cercospora*. While this grouping appears to be the logical one, it is not universally accepted. For this reason, the genus name, *Cercosporella*, will be retained in this paper but the alternate combinations also will be given.

In the Pacific Northwest there are no described species of *Cercospora*-like fungi listed on grasses or cultivated cereals except *Cercosporella herpotrichoides, Cercospora fusimaculans* Atk. and *Scolecotrichum graminis* Fuckel which latter Horsfall has transferred to the genus *Cercospora*, making the combination *Cercospora graminis* (Fuckel) Horsfall (**3**).

Cercosporella Holci sp. nov.[3]

Maculis luteis, vel pallide brunneis, ellipticis, dein confluentibus; margine luteo v. pallide brunneo; myceliis hyalinis v. chlorinis, 0.5–1.2 μ diam., septatis,

[1] Coöperative investigations by the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Oregon and Washington Agricultural Experiment Stations. Published as Technical Paper No. 251 of the Oregon Agricultural Experiment Station.

[2] Associate pathologist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

[3] In the genus *Cercospora* the combination would be **Cercospora Holci** sp. nov.

ramosis, myceliis stromaticis sparsis; conidiophoris carentibus v. brevibus, 5–20 × 1.2–2.5 μ, hyalinis v. chlorinis; conidiis hyalinis, rectis v. curvulis, raro recurvulis, obclavato-filiformibus, pluriseptatis (1–9), 40–105 × 1.5–3.0 μ.
Hab. in foliis *Holci lanati* L.

Spots tan, yellow bordered to darker buff or tawny, elliptical or extensive scald-like lesions on any or all leaves of a plant in mid to late winter and early spring. Mycelia hyaline or faintly chlorine tinted, intracellular and intercellular, 0.5 to 1.2 μ in diameter,

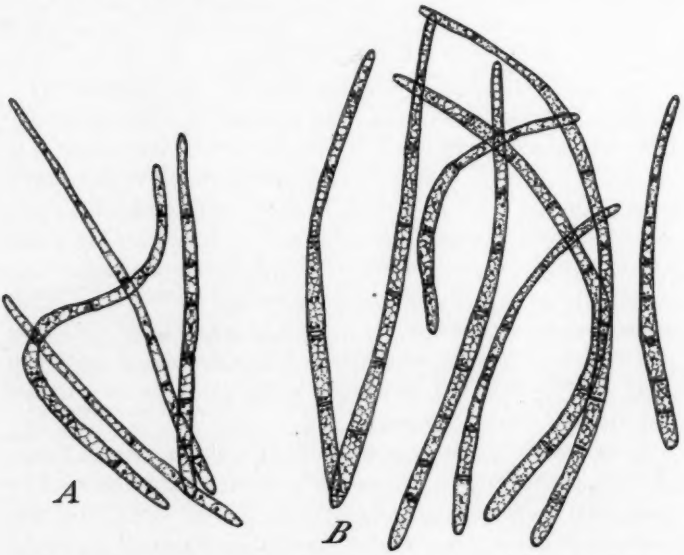

FIG. 1. *A*, conidia of *Cercosporella Holci* from the type collection, Oregon specimen No. *10202*, × 1000; *B*, conidia of *Cercosporella Holci* from Oregon specimen No. *10227*, × 1000; the conidia from this specimen average slightly larger than those from the type collection No. *10202*.

septate, branched; stromatic mycelia loose or nearly absent except subhyaline aggregates in the stomatal cavities and adjacent internal and external leaf parts. Conidiophores frequently but little differentiated from the fruiting stroma, or short, erect, simple or once branched 5–20 × 1.2–2.5 μ, hyaline or faintly chlorine, most common near or in the stomata, sterile hyphae and/or developing spores 10–31 × 1.5–2 μ, mingled with the fruiting hyphae. Conidia hyaline, straight to slightly curved, rarely double reflexed, attached at the larger elliptically rounded base, slender obclavate-

filliform, 1–9 septate, distal end tapering, rounded, contents homogeneous with small globules in the larger cells, 40–105 × 1.5–3.0 μ (FIG. 1, *A* and *B*).

On leaves of *Holcus lanatus* L. in Oregon and Washington.

The following numbered specimens are deposited in the Mycological Herbarium of the Oregon State College:

Collected in Oregon: Alsea Mts. (Benton Co.), *10,202* (Type); Alsea Valley (Lincoln Co.), *10,203* and *10,204;* Near Troutdale (Clackamas Co.), *10,227;* Lobster Valley (Lincoln Co.), *10,274;* East Corvallis (Linn Co.), *10,358;* Fall Creek (Lincoln Co.), *10,404;* Peavey Arboretum (Benton Co.), *10,410.*

Collected in Washington: Bingen (Klickitat Co.), *8089* and *8135.*

In severe cases of infection, *Cercosporella Holci* caused a reaction similar to a scalding or snow injury. The fungus evidently is favored by cold weather, the same as *C. herpotrichoides* (**6**). *Cercorporella Holci* differs, however, from *C. herpotrichoides* in a number of important respects. While *C. herpotrichoides* confines its attack to the culm at the soil line, *C. Holci* causes a leaf spot and rot. The development of dark stromatic (*Cercospora*-like) mycelia in *C. herpotrichoides* is considerable (**6**) while that in *C. Holci* is usually slight and is subhyaline in color (true *Cercosporella*).

Morphologically, the conidia of *Cercosporella herpotrichoides* are distinctly broader and more obclavate than those of *C. Holci.* In pure culture on potato dextrose agar the former differs from *C. Holci* in producing a more compact, mounded type of growth which is of a darker gray color than that of *C. Holci.*

From the fact that *Cercosporella Holci* is not found on any grasses growing in close association with *Holcus lanatus* it is believed that the local host range of this fungus is limited. If it is a recent transfer from a non-graminicolous host its range on other hosts would not be determinable under present conditions inasmuch as there are over 1,000 species of *Cercospora*-like fungi described. Notwithstanding the fact that there are these hundreds of species described, the number on grasses is relatively small, and most of the ones described on this extensive family are robust species entirely distinct from any of the new species found in

Oregon and Washington on grasses. After comparing with all descriptions of grass inhabiting *Cercospora*-like species and with available exsiccati the writer is convinced that *C. Holci* is distinct. W. W. Diehl, Bureau of Plant Industry, U. S. Department of Agriculture, kindly examined the Mycological Collections of the Bureau of Plant Industry at Washington, D. C., and determined that this fungus apparently has no counterpart in that herbarium. Dr. Diehl was of the opinion that the fungus was undescribed.

Cercosporella subulata sp. nov.[4]

Maculis effusis, confluentibus, praelongis, sordide stramineis vel olivaceo-luteis, dein isabellinis; margine fusco, v. luteo-brunneo; myceliis vegetis hyalinis v. chlorinis, septatis, 1–2 μ diam.; myceliis stromaticis olivaceis dein obscuro-nigris; conidiophoris hyalinis v. chlorinis, brevibus, 5–20 $\times$ 1.5–2 μ; conidiis numerosis, hyalinis, subulato-filiformibus, extremo flagelliformibus, raro rectis, fere curvulis, raro recurvulis; conidiis 1–2 septatis, 20–35 $\times$ 2.5–4.3 μ, fere 28–33 $\times$ 2.8–3.5 μ.

Hab. in foliis, et vaginis Melicae subulatae (Griseb.) Scribn.

Lesions extensive, elongate, sometimes diffused as a scald over large portions of the leaves, sordid dull straw color to olive buff and isabelline bordered by darker tones of umber to buffy brown. Vegetative mycelia hyaline to strongly chlorine, septate, branched, 1–2 μ in diameter, stromatic mycelia olivaceous to dull black, aggregated at or near the leaf surface from which are produced short, hyaline to strongly chlorine conidiophores, 5–20 $\times$ 1.5–2 μ. Conidia hyaline, produced in large numbers, borne blunt, broad base down, subulate-filiform tapering to a whip-like distal portion which is sometimes straight, usually curved or flexuous, rarely reflexed. Conidia sometimes 1-septate, usually 2-septate with basal septum at broadest portion of subulate base, sometimes slightly constricted at second septum from base, which usually is at basal end of whip-like distal portion. In old material conidia frequently break at this second septum. Conidia 20–35 $\times$ 2.5–4.3 μ, mostly 28–33 $\times$ 2.8–3.5 μ (FIG. 2, *A*).

On leaves and sheaths of *Melica subulata* (Griseb.) Scribn., Main Divide Trail in Ochoco National Forest, Oregon. Aug. 21, 1916. D. C. Ingram (Ingram B. *606* Oregon State Phanerogamic Collection Sheets No. *23,260* and *23261;* Oregon Mycological Collections *10,669*).

[4] In the genus *Cercospora* the combination would be **Cercospora subulata** sp. nov.

The sperm-like conidium of *Cercosporella subulata* is different from the usual conception of a *Cercosporella* spore. The whip-like distal portion of *C. subulata,* however, is definitely a part of the cytoplasmic structure of the spore and is not a vacuolated or empty appendage such as occurs in *Pseudodiscosia* Host. and Laub., in the apiculated *Dactylella tylopaga* Drechsl. (**1**) or in *Mastigospo-*

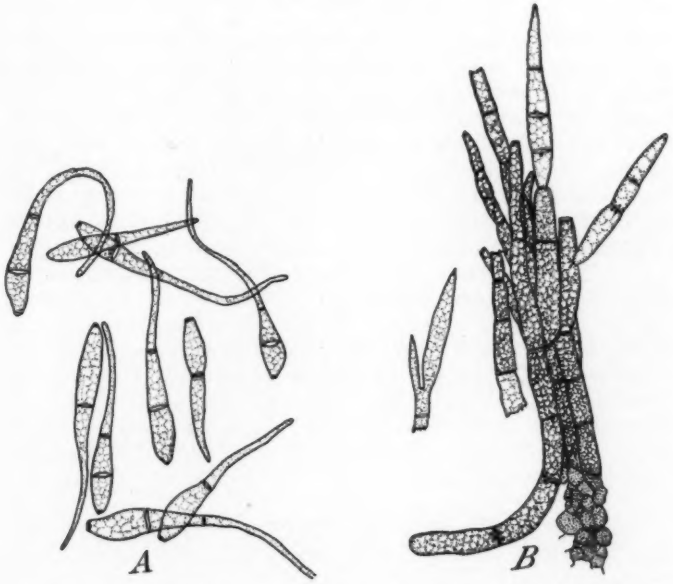

FIG. 2. *A,* conidia of *Cercosporella subulata* from type material, × 1000; *B,* conidiophores and conidia of *Cercospora Bromi* from type material, × 1000.

rium Riess. Solheim (**4**) illustrates species of *Cercospora,* which approach the sperm-like shape of *C. subulata.*

Cercosporella subulata, C. herpotrichoides and the species described later in this paper are the only *Cercospora*-like species known on the tribe Festuceae of the Gramineae. There are no comparable species on the Gramineae, and on this differential basis *C. subulata* is a distinct species.

Cercospora Bromi sp. nov.[5]

Maculis griseis dein sordidis, elongato-striatis, margine fusco v. brunneo; myceliis vegetis variabilibus, hyalinis v. subhyalinis v. fuligineis; myceliis stromaticis olivaceis dein obscuro-nigris; conidiophoris carentibus v. in longitudine variabilibus, simplicibus v. furcatis, singulis v. fasciculatis, fuligineis v. chlorinis; conidiis hyalinis, acrogenis et subacrogenis, obovato-fusiformibus, rectis v. curvulis, 1–4 septatis, 15–45 × 2.5–5 μ (25–43 × 3–4.5 μ); conidiis secundariis 3–15 × 0.8–2 μ, aseptatis.

Hab. in foliis, culmis, vaginis et glumis vivis *Bromi rigidi* Roth.

Spots gray to sordid, elliptical or striate with broader margins dark brown to umber. Vegetative mycelia variable, hyaline to subhyaline to fuligenous, hyphal cells variable in size and shape, rectangular to angular, becoming subspherical or bulbous, stroma dark olive to dull black filling epidermal cells with polygonal and irregular cells and spreading from there to outer surface of the host through stomata. Conidiophores variable in length, sometimes obscure, simple or once branched, single or fascicled to form coremium-like aggregates, fuligenous to chlorine colored (FIG. 2, *B*). Conidia hyaline, borne singly or in pairs, acrogenously or subacrogenously, obovate-fusiform, slightly curved, 1–4 septate, slightly constricted at septa, base blunt with prominent scar or hilum, 15–45 × 2.5–5 μ (mostly 25–43 × 3–4.5 μ; secondary conidia produced singly on from 25 to 80 per cent of the conidia, usually from the second or less commonly from the first basal cell, deflected at 45 degree usually toward the leaf base, slender fusiform, 3–15 × 0.8–2 μ (mostly 10–12 × 1.5–1.8 μ), non-septate, semi-deciduous (FIG. 3).

On leaves, sheaths, culms and glumes of *Bromus rigidus* Roth, near Tumwater, Wasco Co., Ore. (*10,405* (Type)) and at Corvallis, Ore. (*10,751*).

This fungus was collected in the semi-arid sandy reaches along the Columbia River in eastern Wasco County, Ore., in March 1935, and in a vacant lot in the semi-humid region at Corvallis, Ore., in May 1936. In the abundant material at Corvallis the disease was characterized by the gray, striate lesions which produced an anthracnose-like superficial lesion on the various green parts of the plants. While very superficial on the silicified culm parts, the fungus apparently caused considerable stunting, particularly where the attack had reached the heads. The disease is of no

[5] In the genus *Cercosporina* the combination would be **Cercosporina Bromi** sp. nov.

economic importance; in fact, this grass, which is called ripgut, is the least desirable of the cheat grasses, which generally are troublesome weeds in the Pacific Northwest.

The secondary conidia of *Cercospora Bromi* are somewhat different from those on any other fungus seen by the writer. Because they are semi-deciduous, they are considered secondary co-

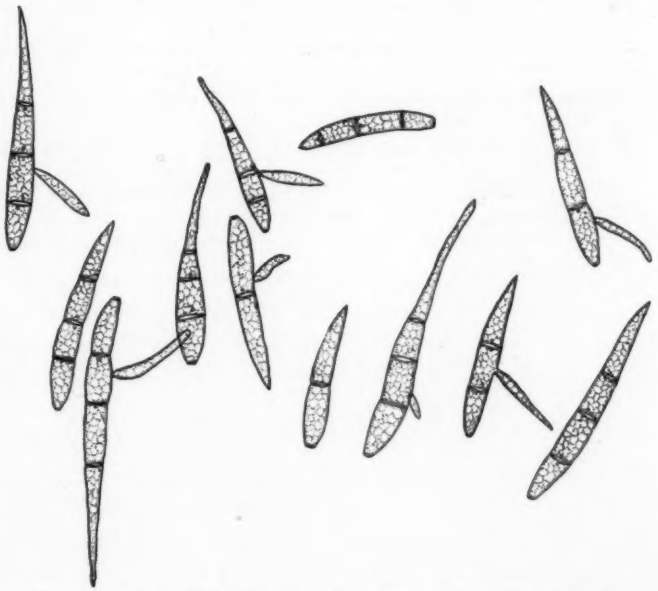

FIG. 3. Conidia of *Cercospora Bromi* from type material, × 1000.

nidia rather than germ tubes, although the distinction between these in other *Cercospora*-like fungi is sometimes not great. For example, Foëx and Rosella (**2**) mention secondary conidia in *Cercosporella herpotrichoides* and Sprague and Fellows (**6**) have seen these and illustrated them but make the notation that they almost immediately germinate again. In *Cercospora Bromi,* germination is apparently definitely arrested. These secondary conidia are not cilia such as occur in *Pseudodiscosia Avenae* Sprague and Johnson (**7**). The chlorinous nature of the coloring and the type of appendage removes it from *Mastigosporium* Riess. While it is rec-

ognized that the regularity of the formation of the conidia is remarkable, it seems clear that the fungus belongs in the genus *Cercospora*.

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A NEW SPECIES OF CANDELOSPORA CAUSING DECAY OF CITRUS FRUITS [1]

H. S. Fawcett and L. J. Klotz

(with 6 figures)

In January, 1932, at Citra, Florida, a new fungus was isolated from a decayed portion of an orange fruit. A firm type of decay had started on one side. The rotted region was slightly sunken, with a definite margin, brown on the exterior, and brownish through the albedo and for a short distance inward on the divisions between the segments.

Inoculations made in injuries in the rind of oranges, lemons, and grapefruit resulted in decay similar to that from which the fungus was isolated. The fungus was again isolated from the decayed portion of these inoculated fruits.

TYPE OF DECAY PRODUCED BY INOCULATIONS WITH CANDELOSPORA

Although the rate of decay varied with the temperature, the type of decay was similar at most of the temperatures within a certain range. The following is a description of the decay as it appeared at 20° C. and a relative humidity of about 85 per cent. On mature Valencia orange fruits taken directly from the tree and inoculated in September, the decay spots, after 4 weeks (fig. 1), had definite margins, were firm, ans were cinnamon brown to Prout's brown in color.[2] On cutting, the entire thickness of the rind was found discolored and part of the pulp affected but not yet discolored. At temperatures a few degrees higher the divisions of the segments and core showed light-colored mycelium.

[1] Paper No. 352, University of California Citrus Experiment Station and Graduate School of Tropical Agriculture, Riverside, California.

[2] Color nomenclature throughout this article from: Ridgway, Robert. Color standards and color nomenclature. 43 p. 53 colored plates. Published by the author, Washington, D. C. 1912.

At 20° C. inoculations on light green lemons direct from the tree developed decay which, externally, was mars brown in color. The rind, pulp divisions, and core were dark brown to black (FIG. 2). The pulp, exclusive of divisions, was usually not discolored. At higher temperatures the external diseased areas were smaller, but

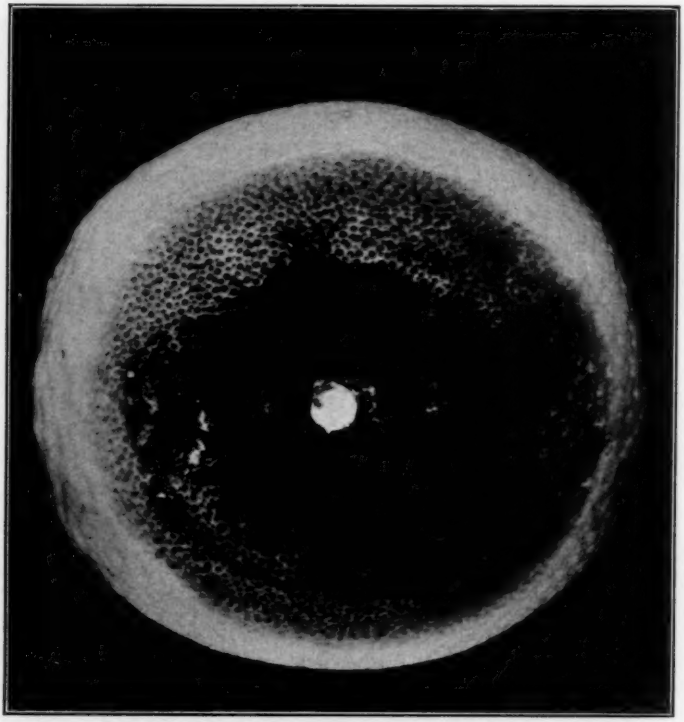

FIG. 1. Decay in Valencia orange produced by *Candelospora Citri* in a 4 weeks' incubation at 20° C.

on cutting, the discoloration was found extending around the inside of the rind on the divisions and throughout the core. This internal discoloration (FIG. 2) was similar in extent but darker and firmer than typical center rot of lemons due to *Alternaria Citri*.

Microscopic examinations in both oranges and lemons showed hyphae of the fungus to be abundant in the stalks of the juice sacs but not present on or in the juice sacs themselves. This was possibly due to the inability of the fungus to grow at the H-ion concentration of the lemon and orange juice.

EFFECT OF VARIOUS TEMPERATURES ON GROWTH AND PATHOGENICITY OF THE FUNGUS [3]

Transfers with mycelial disks 4 mm. in diameter were placed on glucose-potato agar in the center of petri dishes. Five dishes

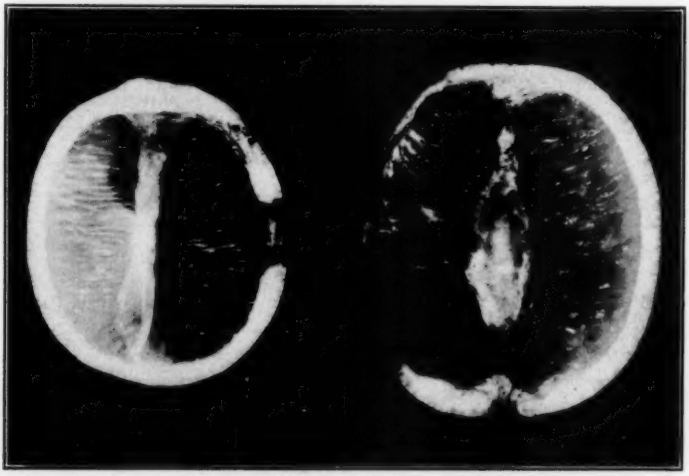

FIG. 2. Internal decay of lemon and Valencia orange caused by *Candelospora Citri.*

were placed in each of seven constant-temperature cabinets. The temperature of any one cabinet varied less than ½° C. from the mean. The rate of invasion of citrus fruit by the fungus at the several temperatures was also studied. Orange and lemon fruits were inoculated by placing a drop of spore-mycelium suspension in each of 2 uniform wounds made on opposite sides of the rind

[3] The authors wish to acknowledge the assistance of L. L. Huillier in carrying out these experiments.

at the equator. The wounds were made by removing from the rind with a cork borer a disk 4 mm. in diameter and 2 mm. deep. One of the two inoculations was covered with a small piece of adhesive tape. The fruits were then wrapped in packing tissue and placed in paper bags.

One set of inoculations was made on August 28, 1935, and the other on November 9, 1935. Since the results of these two sets appeared to fit fairly well into one series, they are combined here into one table (table 1). Two measurements of the diameter of

TABLE 1

DIAMETER, IN MILLIMETERS, OF MYCELIAL DISK AND DECAY LESIONS DUE TO INOCULATIONS WITH *Candelospora Citri*

Temperature, °C.	Mycelial disk in 6 days	Decay on yellow lemons in 21 days	Decay on green lemons in 22 days	Decay on mature Valencias in 22 days	Decay on green Valencias in 22 days
7.7 *	0.0	——†	0.0	0.0	——
11.8	21.9	24.1	12.7	21.5	13.2
14.0 *	24.0	——	17.0	21.5	——
17.3	42.2	28.4	11.8	26.1	24.7
19.8 *	45.0	——	12.0	36.2	——
21.5	52.1	40.6	9.1	35.3	31.7
24.0 *	53.0	——	9.4	41.6	——
25.5	58.2	45.0	8.6	36.3	10.4
27.5 *	37.0	——	6.0	29.5	——
29.3	41.1	9.9	6.2	21.9	9.3
30.4 *	15.2	——	6.2	7.0	——
35.0 *	0.0	——	0.0	0.0	——

* Average temperatures used to obtain data during the period beginning August 28, 1935. The remaining temperatures were used in the experiment begun November 9, 1935.

† —— indicates no test made at this temperature.

each mycelial growth were made and the average taken. The average of the readings of the 5 petri-dish cultures, which includes the initial 4-mm. disk, are recorded in the table for the first 6 days. Similarly the diameters of the fruit lesions were also measured and the averages for the 22-day period of incubation recorded. This interval of 22 days was chosen in order to obtain a common time interval for combining the 2 tests. The temperature relation as a whole was about the same for all time intervals recorded.

Table 1 indicates that the optimum temperature for growth on glucose-potato agar is near 25.0° C., that the minimum is above

7.7° C. but below 11.8° C., and that the maximum is above 30.4° C. but below 35.0° C. A similar relation is indicated for rate of decay on mature lemon and orange fruits. For green fruits, however, the rate of decay is not only lower at all temperatures tested, but there is evidence that the optimum temperature for the development of lesions is much lower than that for mature fruits. The largest surface lesions on green lemons developed at 11.8° and 14.0° C., and those on green Valencias at 17.3° and 21.5° C.

THE CAUSAL FUNGUS

A study of this fungus shows that it belongs to the genus *Candelospora* erected by Hawley (2). As far as known no other species of this genus has been described. This was described as *Candelospora ilicicola* Hawley on leaves of *Ilicis aquifolium* in Ireland. The genus, according to Hawley, "differs from *Mucrosporium* in its penicillate branching and in its conidia produced singly at the tips of the branchlets." [1]

The *Candelospora* species on citrus differs from *C. ilicicola* Hawley in having smaller spores, in having fawn to vinaceous fawn-colored mycelium instead of white tufts, and in the lack of mucus in the heads. The citrus species has in some cultures a characteristic torch- or beacon-like hypha 240 to 275 μ in length which terminates in a swollen end. The end is usually elliptical in shape but may have several other forms (FIG. 3). Usually one conidiophore, occasionally more, develops on this structure at a point 60 to 90 μ from the union with the mycelium. Rarely an aerial torch-like hypha is found that bears no fruiting body. Oc-

[1] The genus *Cylindrocladium* first described by Morgan (4) in 1892 resembles the genus *Candelospora*. However, the figure of *Cylindrocladium scoparium* by Morgan shows 2-celled (1-septate) spores and a fruiting structure exclusive of spores having a system of branches numbering 6 divisions in some instances. *Candelospora Citri* usually has 4 such divisions. The monopodial club-like or beacon-like hyphae, which we have seen in some cultures of *Candelospora*, were not described or figured by Morgan for *Cylindrocladium*.

Both Massey (3) and Anderson (1) found that *Cylindrocladium* spp. have the club-like hyphae and their figures and descriptions otherwise closely resemble *Candelospora Citri*. The conidium of *Cylindrocladium* spp., however, has but two cells.

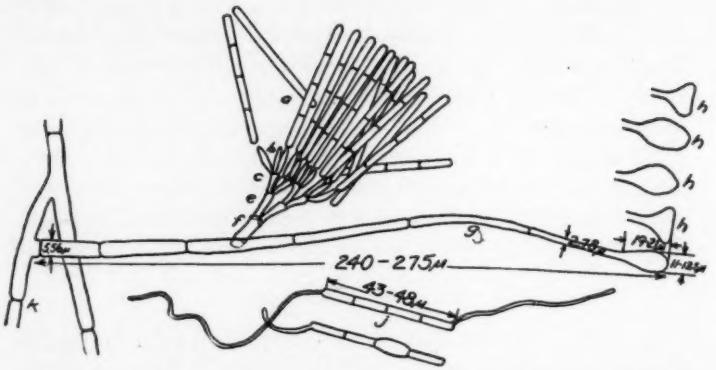

FIG. 3. Tracing of photomicrograph of *Candelospora Citri* showing morphology of fructification: *a*, conidia; *b*, sterigmata; *c*, rami; *e*, metulae; *f*, conidiophore; *g*, torch-like structure with swollen end cell (*h*); *j*, germinating conidia.

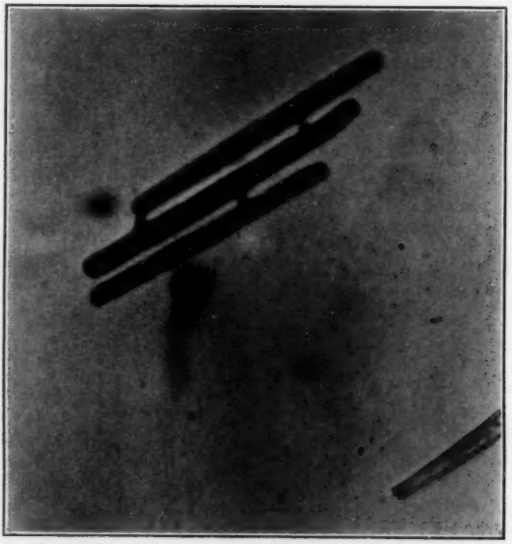

FIG. 4. Photomicrograph of germinating conidia of *Candelospora Citri* showing beginning of anastomosis, × approx. 1000.

casionally a mount shows a beacon branching laterally from another beacon, the former bearing the conidiophores and conidia. Fructifications also arise directly from the mycelium in the absence of beacons.

The hyphae anastomose freely. The spores begin to germinate either from the end (FIG. 3) or, if lying side by side, may germinate laterally, the germ tubes anastomosing with other spores (FIG. 4). When grown on glucose-potato agar in petri dishes for 9 days at room temperatures of 17° to 21° C., the fungus produced abundant fluffy, cottony growth both in the media and in the air (FIG. 5). Viewed from the top, the color was matched with

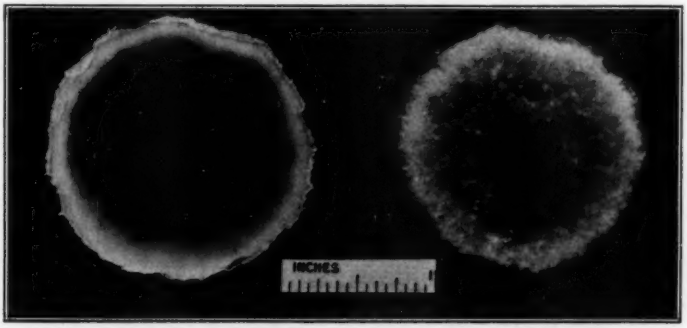

FIG. 5. Colonies of *Candelospora Citri* on dextrose-potato agar. Left, bottom view; right, top view.

Ridgway's cinnamon rufus to hazel; seen from the under side the color was found to vary from hazel to Kaiser brown to hay russet and liver brown.

The genus *Candelospora* is described by Hawley as follows:

Hyphae steriles repentes. Conidiophoris erectis, septatis, hyalinis, irregulariter ramosis vel etiam simplicibus, supra penicillatim divisis. Conidiis singulis in *ultimis* ramulis ortis, hyalinis, multiseptatis.

Candelospora Citri sp. nov.

Conidiophores 14 to 23 μ in length, occurring singly or severally on a torch-like hypha which terminates in a swollen cell. Fructification beyond the conidiophore 83 to 93 μ, divided into rami, metulae, sterigmata, and conidia (FIG. 3). Conidia triseptate,

cylindrical, obtuse at the ends, 43 to 48 × 4.1 to 4.8 μ. Torch-like projection 240 to 275 μ long and 5 to 6 μ wide at base and 2.5 to 3 μ wide at narrowest point, with swollen ends 19 to 21 × 11 to 12.5 μ (FIG. 6). No mucus was observed on citrus fruits or on glucose-potato agar.

Habitat.—On decaying fruits of *Citrus sinensis* Osbeck in Florida, U. S. A. Described from cultures on glucose-potato agar,

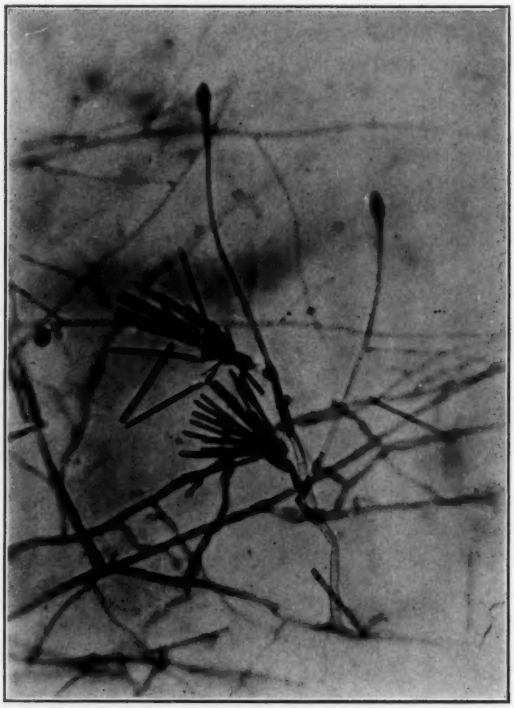

FIG. 6. Photomicrograph of *Candelospora Citri,* × approx. 300.

dried specimens of which are deposited at the Citrus Experiment Station, Riverside, California; at the United States Department of Agriculture, Washington, D. C.; at the Department of Botany, Harvard University; at the British Museum, South Kensington, England; and at the Imperial Bureau of Mycology, Kew Gardens, Richmond, England.

SUMMARY

A decay of citrus fruits caused by a new species of *Candelospora* was found on an orange fruit at Citra, Florida, in 1932. Inoculations showed the fungus capable of decaying lemons, oranges, and grapefruit, mature fruit being more susceptible than immature fruit.

The fungus grown on glucose-potato agar has an optimum temperature for growth near 25.0° C., a minimum between 7.7° and 11.8° C., and a maximum between 30.4° and 35.0° C.

This fungus differs from *Candelospora ilicicola* Hawley, apparently the only other species of the genus described, in having smaller spores, in having fawn to vinaceous-colored mycelium instead of white tufts, in the lack of mucus in the heads, and in having peculiar aerial hyphae which terminate in swollen cells. Conidiophores grow out laterally from these beacon-like hyphae.

The fungus is described as a new species under the name *Candelospora Citri.*

UNIVERSITY OF CALIFORNIA CITRUS EXPERIMENT STATION,
RIVERSIDE, CALIFORNIA

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A FUNGUS ON LACE BUGS

VERA K. CHARLES

(WITH 2 FIGURES)

In March of 1935 specimens of lace bugs (*Leptopharsa Heveae* Drake & Poor) on *Hevea brasiliensis* from Para, Brazil, were sent by Dr. C. H. T. Townsend through the Bureau of Entomology and Plant Quarantine to the Division of Mycology and Disease Survey, of the Bureau of Plant Industry, for the determination of a fungus thought to be parasitic on the insects. Observations made by Dr. Townsend indicated that the fungus had been fairly effective in controlling the lace bug which had been causing some concern as a rubber plant pest. He stated that a considerable percentage of the immature stages of the insect had been attacked and killed. Later, Dr. J. R. Weir sent additional specimens of the same insects attacked by this fungus, with the information that the fungus had practically destroyed the bugs over wide areas in certain of the localities where rubber plantings had been made. Additional specimens have been received from time to time from Dr. Weir who has made extensive field observations.

In examining this material it was found that the mycelium of the fungus was especially well developed on the ventral surface of the host (FIG. 1 *A*) and that the bugs were attached to the lower surface of the leaves by irregularly digitate rhizoids (FIG. 2 *C*). Instances were observed in which these became septate and the divisions rhomboid and thick walled forming a subiculum-like mass. With a hand lens the fungus covering the insects appeared white to grayish white or pale yellowish in old specimens, which had apparently been attacked when young, and their development inhibited by the presence of the fungus.

In the early stages the mycelium was fine, hyaline and septate, and resembled a net covering the insects. The older hyphae from which the conidiophores developed were fuliginous and more closely septate, and about 8 μ in diameter. While the majority of

the hyphae were found to occur singly, strands consisting of 5–12 closely associated, parallel hyphae were also observed. Though no true clavae were found, the strands were sufficient, however, to make evident the stilbaceous nature of the fungus.

The fungus was found in all specimens to be fruiting very sparingly, but it exhibited a remarkable variation in the arrangement of the phialides. In the majority of cases the fructification was seen to be irregularly verticillate, whorls of 3–6 flask-shaped phialides being most common, although instances of opposite phialides were also observed. In addition to these two types of phialide development single phialides were often found occurring at irregular intervals along the mycelium (FIG. 2 *A* and *D*). This particular type suggested the genus *Hirsutella* which in addition to the conidial fructifications on clavae, also develops phialides on individual hyphae. The phialides are flask-shaped, and terminated by a long, filiform sterigma bearing a single oblong spore, but apparently pip-shaped, due to the gelatinous substance which surrounds them. Occasionally a second phialide will develop from the basal cell of the original phialide and produce a spore. Still another variation was observed in which a second sterigma developed from the upper part of the phialide (FIG. 2 *E*). While the wall of the phialide is ordinarily entire in outline, an occasional phialide or group of phialides would be observed with small protuberances on the wall (FIG. 1 *B* and 2 *B*), about 4 μ in length. These abnormal phialides were never observed to develop conidia. Because of the marked variation exhibited by the fungus in the method of fruiting it is difficult to suggest a genus sufficiently flexible to include a species presenting such diverse morphological variations in the method of spore production.

In referring to Petch's papers on entomogenous fungi, two species have been noted, *Verticillium hemipterigena* and *Cladobotryum ovalisporum*, which show certain affinities with the fungus on the lace-bug. The first of these was described by Petch[1] in 1932 from material of leaf hoppers on bamboo from Ceylon. This was stated to be the conidial stage of *Torrubiella hemipterigena* Petch. The conidial form of this fungus bears a close resemblance

[1] Petch, T. Notes on entomogenous fungi. Trans. Brit. Myc. Soc. **16**: 236. 1932.

to the species under consideration, but differs in the size and shape of the conidiophores, phialides and spores. The conidiophores are described as up to 150 μ long, and 1.5 μ in diameter, while the

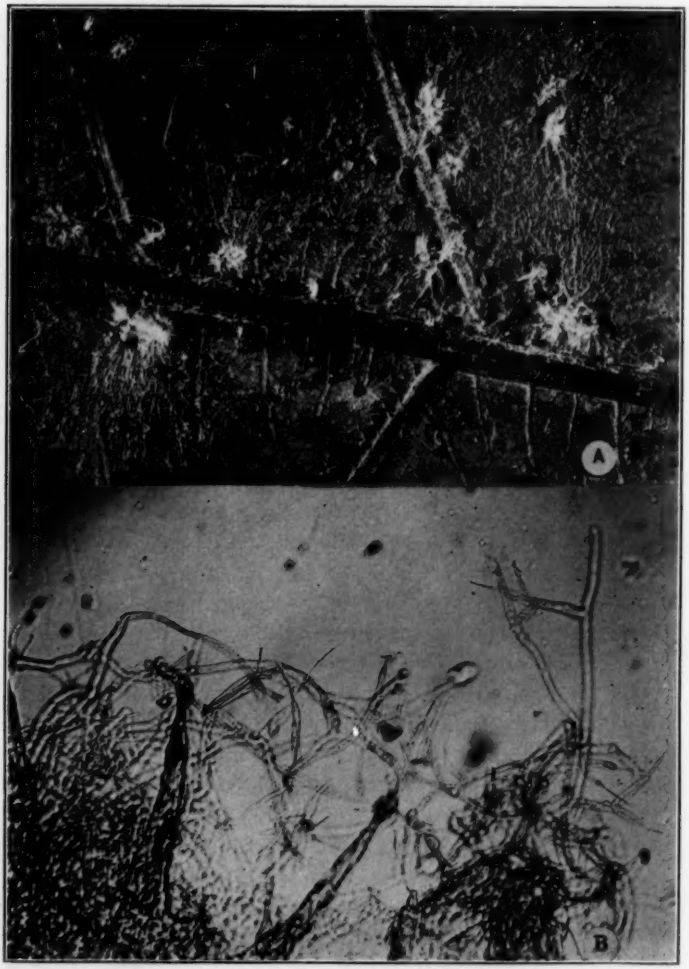


FIG. 1. *A*, part of rubber leaf showing lace bugs attacked by *Hirsutella verticillioides*, × 5; *B*, photograph of hyphae showing phialides with projections on the walls, × 250.

conidiophores of the lace bug fungus range from 100–450 μ in length.

The phialides of *Verticillium hemipterigena* are described as 12–16 μ long, 1 μ diameter below, the conidia narrow-oval or fusoid or sub-falcate, ends acute hyaline continuous 5–8 $\times$ 1 μ usually solitary, occasionally two in a parallel bundle. In the species on lace bugs the flask-shaped portion of the phialides is 4–7 $\times$ 20–28 μ. The spores are 4–5 $\times$ 6–8 μ.

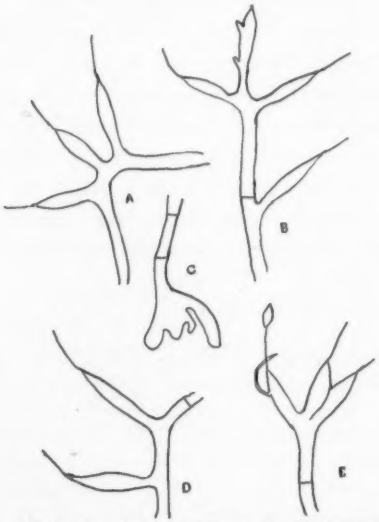


FIG. 2. Camera lucida drawings of *Hirsutella verticillioides* showing, *A* and *D*, development of single phialides; *B*, phialide showing protuberances on the wall; *C*, rhizoids; *E*, development of a second sterigma on the phialide. $\times$ 350.

In the same paper Petch (l. c., p. 230) described the second fungus *Cladobotryum ovalisporum* from material on frog hoppers from Ceylon. This fungus is described as having conidiophores up to 320 μ high, 2 μ in diameter below, the phialides up to five in a whorl, narrow flask-shaped, 5–8 μ long, 1–1.5 μ in diameter below and bearing minute cylindrical sterigmata, usually simple, sometimes bifurcate, toward the apex. While the sterigmata suggest the projections (FIG. 1 *B* and FIG. 2 *B*) on the phialides of the

fungus studied here, they are much smaller and more cylindrical. The conidia are also shorter ranging from 3–5 × 1.5–2 μ.

The fungus on lace bugs is remarkable for the great variation in the size of its conidiophores and phialides. Fruiting is more abundant in the young growing area, but the conidiophores are smaller, more flexuous and graceful than those developed from the older hyphae. In the latter case the conidiophores are stouter, erect, rigid and with fewer whorls of phialides.

In view of the striking variations exhibited by the lace bug fungus its taxonomy is debatable. The shape of the phialides, the long sterigma and the character of the spores suggest the genus *Hirsutella,* but the verticillate arrangement of the phialides is characteristic of *Verticillium* and *Cladobotryum.* However, our study of the available material leads us to the conclusion that the organism has more affinities with the genus *Hirsutella* than with *Verticillium* or *Cladobotryum* and we are therefore designating it *Hirsutella verticillioides* with the following description:

Hirsutella verticillioides sp. nov.

Mycelium producing a film over the insects, white later pale yellow, hyphae erect or decumbent, branched, older coarse thick-walled 6–10 μ in diameter, short septate, pale fuliginous, 5–12 fasciculate; conidiophores erect, rigid, simple, septate, tapering, hyaline above, fuliginous below, 100–450 μ in height; phialides 3–6 verticillate, flask-shaped, 4–7 × 20–28 μ, attenuated into a sterigma 8–15 μ in length; conidia apical, hyaline, oval but apparently pip-shaped due to a gelatinous substance which surrounds them, 4–5 × 6–8 μ; rhizoides digitate sometimes septate, cells becoming thick-walled and rhomboid.

Mycelio insectum membrana obducente, primo albo dein pallide flavido, hyphis repentibus v. erectis, septatis, ramosis, vetustis muris incrassatis, 6–10 μ diam. breve septatis, pallide fuligineis, 5–12-fasciculatis; conidiophoris erectis, rigidis, simplicibus, septatis, attenuatis, infra fuligineis, supra hyalinis, 100–450 μ alt.; phialidibus 3–6-verticillatis vel singulis, lageniformibus, 4–7 × 20–28 μ, sterigmati 8–15 μ longo; conidiis acrogenis, hyalinis, ovalibus, propter substantiam gelatinosum circumvallentem pseudolimoniformibus, 4–5 × 6–8 μ; rhizoidibus digitatis, interdum septatis, cellulis rhomboideis, muris incrassatis.

In *Leptopharsam Heveae* in foliis *Heveae brasiliensis,* in Brasilia.

Type: Communicated by A. Johnston, Para, Brazil, July, 1935, in Mycological Collections of the Bureau of Plant Industry, No. *70902*. Portions of this collection also deposited in the Farlow Herbarium of Harvard University, the University of Michigan Herbarium, and the New York Botanical Garden Herbarium. Additional collections (Myc. Coll. Nos. *70903, 70904*) received from J. R. Weir, Para, Brazil, April 8 and May 14, 1936.

BUREAU OF PLANT INDUSTRY,
U. S. DEPARTMENT OF AGRICULTURE,
WASHINGTON, D. C.

A NEW SPECIES OF MAGNUSIA [1]

L. M. AMES

(WITH 14 FIGURES)

The interesting fungus considered and illustrated in the present note is a dung-inhabiting member of the Aspergillaceae. The substratum, horse dung, upon which it was first found by the writer, was collected in the spring of 1934 in The Great Smoky Mountains in Tennessee on Gregory Bald, near the North Carolina State line.

For nearly two years pure cultures have been maintained on agar media containing a small amount of potato broth and horse-dung extract. On the same media pure cultures of *Magnusia nitida* [2] have been grown for the purpose of comparison. Observations and comparisons of both species have been made under similar growing conditions.

If one disregards the conspicuous appendages of *Magnusia nitida* and *Magnusia* sp. described below, it is at once apparent that in size and shape the perithecia of both species are indistinguishable, and indeed their asci, spores, and conidia are similar in size and color. However, even a macroscopic examination will suffice to distinguish the two species when their prominent appendages are taken into consideration. The copious, elevated clusters of elongate appendages give the perithecia of *M. nitida* a distinctly bushy appearance, while in contrast the fewer, much shorter, and more regularly coiled appendages decorate the perithecia of the latter species in a much less showy manner. The conspicuous differences in the length of appendages, the amount of their coiling, and the average number produced, convinced the writer that the fungus herein described is quite distinct and worthy of designation

[1] Contribution from the Laboratories of Cryptogamic Botany and the Farlow Herbarium, Harvard University No. 143.

[2] Cultures obtained from Mr. Herman R. Sweet of the Biological Institute, Harvard University.

as *Magnusia brachytricha;* the specific name referring to the short, rigid hairs ornamenting the perithecia.

Magnusia brachytricha sp. nov.

Perithecia superficialia, non ostiolata, oblonga, obtuse triangularia vel quadrangularia, 125–325 × 100–225 μ, nigra, primum pilis longis tenuibus flexuosis pallidis circiter 175 × 2 μ maturitate evanescentibus aequaliter vestita, in uno vel pluribus locis pilis conspicuis rigidis circinatis septatis lucide nigris 1–5-fasciculatis 5–8 μ latis et 50–150 μ longis e cellulis interioribus parietis manifeste cellularis orientibus ornata. Asci numerosi, globosi (10–13 μ diam.) vel oblongo-pyriformes (18–22 × 10.5–12.5 μ), sporis ellipsoideis, utrinque acutis 6–8 × 3–5 μ primum hyalini, demum dilute flavo-fusci. Conidia ellipsoidea, utrinque acuta, 4.5–5 × 2.2–2.5 μ, coremio insidentia.

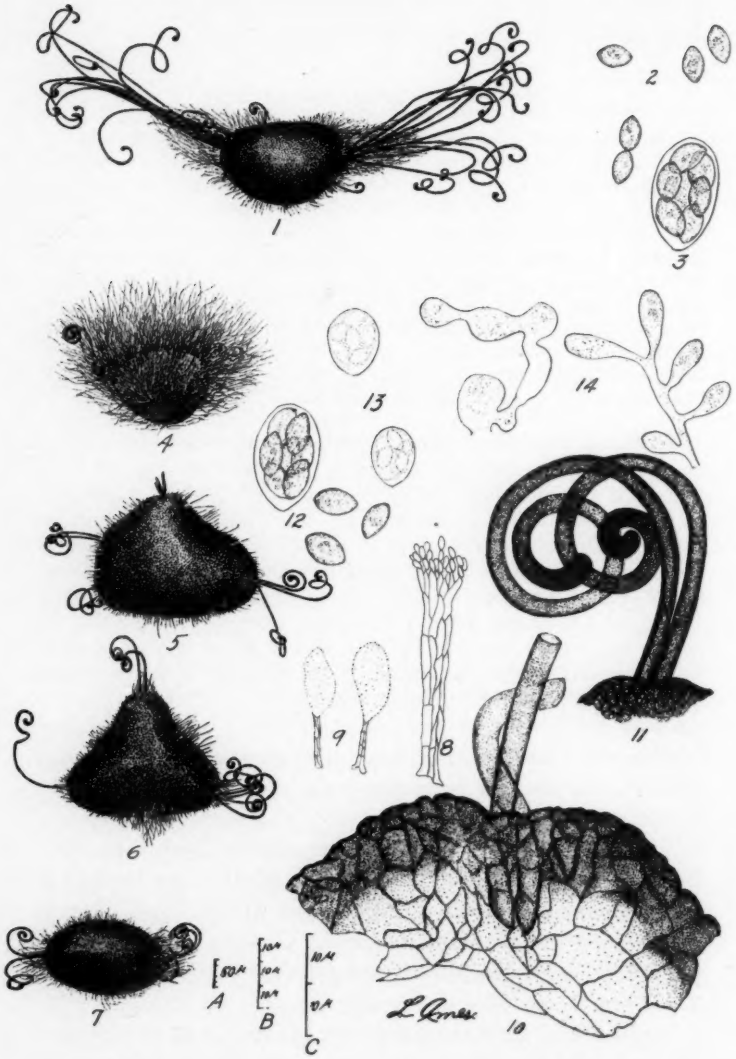
Magnusae nitidae Sacc. arcte affinis sed pilis circinatis multo brevioribus diametrum perithecii non aequantibus facile distinguitur.

Perithecia superficial, non-ostiolate, oblong, obtuse triangular or quadrangular, 125–325 × 100–225 μ, black, at first covered with long, flexuous, light-colored hairs about 175 × 2 μ which disappear at maturity. Perithecia at one or several points ornamented with conspicuous, stiff, shiny-black circinate, septate hairs 1–5-fascicled 5–8 μ wide and 50–150 μ long, originating from internal cells of the perithecial wall. Asci numerous, globose (10–13 μ diam.) to oblong-pyriform (18–22 × 10.5–12.5 μ), 8-spored, spores ellipsoid, acute at both ends, 6–8 × 3–5 μ, at first hyaline, becoming dilute yellowish-brown. Conidia ellipsoid, acute at both ends, 4–5 × 2–2.5 μ, borne on a coremium.

Magnusia brachytricha is easily distinguished from *M. nitida* Sacc. by the circinate hairs which are usually shorter than the diameter of the perithecium.

Type: pure cultures deposited in the Farlow Herbarium, Harvard University. Pure cultures also deposited in the Herbarium of the Division of Mycology and Disease Survey, U. S. Department of Agriculture, Washington, D. C. The species was developed on horse dung which was collected on Gregory Bald in The Great Smoky Mts. in Tennessee.

In the fall of 1935 the writer was given a small portion of a *Magnusia* which had been cultivated by Dr. W. W. Diehl of the Division of Mycology and Disease Survey, U. S. Department of Agriculture, Washington, D. C. This particular *Magnusia,* iden-



FIGS. 1–3, *Magnusia nitida;* 4–14, *Magnusia brachytricha.*

tical with *M. brachytricha,* came from bear dung which was collected on Grandfather Mountain, North Carolina, in June 1927. A small portion of this material which the writer placed in an agar plate produced mycelia after six or seven days and matured perithecia about two weeks later. The delay in growth and fruiting was probably due to the extremely desiccated condition of the herbarium specimen, for subsequent transfers came into fruiting much sooner. It is interesting to note that Diehl's specimen was able to revive and fruit after having been in the herbarium for about seven years.

In addition to the collection made by Diehl in 1927, evidence of a still earlier collection of *Magnusia brachytricha,* but recognized only as *M. nitida,* is obtained from figures in Mycotheca Marchica, Zoph and Sydow, No. *100;* the central figure is unquestionably that of *M. nitida,* but the other two perithecia illustrated certainly resemble *M. brachytricha.*

In conclusion I wish to thank Dr. William W. Diehl for the herbarium specimens of his 1927 *Magnusia* isolation. I am particularly grateful to Mr. Alfred Rehder for giving me valuable assistance in the writing of the Latin description, and to Dr. William H. Weston for his interest and helpful criticism.

EXPLANATION OF FIGURES

Fig. 1–3, *Magnusia nitida:* 1, Mature perithecium showing long, circinate appendages and remnants of flexuous hairs; illustrated here for comparison with *M. brachytricha;* 2–3, mature ascospores and ascus with contained ascospores. Fig. 4–14, *Magnusia brachytricha:* 4, young perithecium covered with long, flexuous, light-colored hairs; 5–7, mature perithecia showing variation in shape and size; note also remnants of flexuous hairs (the perithecia illustrated were grown on agar and therefore retain considerable fragments of the flexuous hairs; on dung these hairs wholly disappear); 8, a coremium showing colorless conidia at apex; 9, conidial clusters borne on coremia; 10, section showing origin of ornamental hairs from internal cells of the perithecial wall; 11, detail of rigid, ornamental hairs showing septations; 12, mature ascus and ascospores; 13, two immature asci containing undeveloped ascospores; 14, stages in the development of ascogenous hyphae drawn from fresh material which was slightly stained with basic methylene blue. Fig. 1, 4–7, and 9 drawn to scale A; fig. 8 and 11 drawn to scale B; fig. 2–3, 10, and 12–14 drawn to scale C.

SOME SPORANGIAL VARIATIONS IN SAPROLEGNIA FERAX

FRED T. WOLF

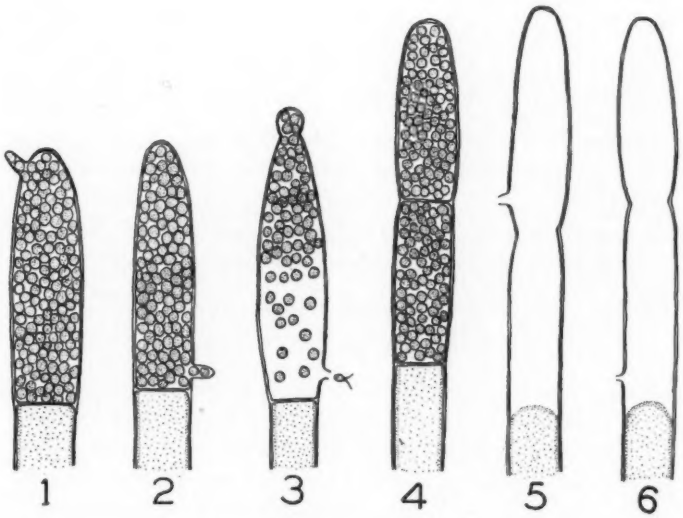
(WITH 6 FIGURES)

It is probably because generic distinctions within the Saprolegniaceae are based largely on sporangial characters that attention has been directed toward morphological variations in these structures. Horn (**3**) induced the production of both terminal and intercalary sporangia with lateral openings in *Achlya polyandra* by the use of high temperatures and dilute solutions of metallic salts. Lechmere (**4**) states: "As the result of keeping a species of *Saprolegnia* under observation for a period of five months it has been possible to obtain on the same mycelium the methods of asexual reproduction which are characteristic of six different genera." Similar results were obtained with an undetermined species of *Saprolegnia* by Collins (**2**), who regards her fungus as being probably identical with *S. Thureti* deBary (*S. ferax* (Gruith.) Thuret). Coker (**1**) points out, however, that such resemblances to other genera are merely superficial, and do not imply, as Lechmere maintained, that the accepted classification of the group is invalid. Coker also enumerates the sporangial variations which have been reported for a considerable number of species.

The occurrence of two sporangia, one below the other, terminating the hyphal tips of *S. ferax* was described by Lechmere (**5**) in 1911, but has apparently not been mentioned by subsequent authors. This rather rare abnormality was recently observed in hempseed cultures of two strains of *S. ferax,* one isolated on corn grains from tanks in the greenhouse of the Biology Building, University of Wisconsin, on January 15, 1936, and the other collected at the Limnological Laboratory at Trout Lake, Wis., on July 22, 1936. Although the majority of the sporangia produced were of the primary type described as being typical of this species, in about 10 per cent of the cases secondary sporangia were formed

(FIG. 4), their development being of the basipetal type of Lechmere.

Several instances were noted (FIG. 1–3) in primary, apparently typical sporangia in which the papilla through which the zoöspores emerged was formed at various points on the lateral walls of the sporangium rather than at its tip. Such abnormally located papillae were more frequently observed near the base of the sporangium (FIG. 2) than elsewhere.



FIGS. 1–6. Sporangia of *Saprolegnia ferax*.

Lechmere (**5**) figured the discharge of both the terminal and subterminal sporangium as occurring by means of a terminal papilla in the terminal sporangium, the subterminal sporangium discharging its zoöspores following dissolution of the wall separating the two sporangia. The variations noted above concerning the place of papilla formation in primary sporangia were also observed in secondary sporangia (FIG. 5, 6). In some cases the subterminal sporangium was the first to discharge.

Apparently, therefore, in *S. ferax* sporangia may be produced and discharge in a number of different ways, which are probably determined, at least in part, by external conditions.

The writer wishes to express his appreciation to Dr. E. M. Gilbert for his advice and criticisms in connection with this work, which was supported by a grant from the Wisconsin Alumni Research Foundation.

UNIVERSITY OF WISCONSIN,
MADISON, WIS.

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NEW ZOOPAGACEAE DESTRUCTIVE TO SOIL RHIZOPODS

CHARLES DRECHSLER

(WITH 6 FIGURES)

Three species of *Cochlonema* and one species of *Zoopage* are newly described herein, increasing the recorded membership of the Zoopagaceae from 22 to 26. Observations on sexual reproduction in two of the new species of *Cochlonema* now remedy in some degree the inadequacy of detail in the knowledge hitherto available on zygospore formation in that genus. Biological interest attaches perhaps more especially to the other two forms, inasmuch as they subsist on testaceous rhizopods, rather than on *Amoebae* or nematodes, which between themselves provide the source of nourishment for all organisms previously assigned to the family.

COCHLONEMA ODONTOSPERMA

A fungus conspicuous among conidial Phycomycetes for the distinctive sculpturing of its zygosporangia was observed in more than a dozen old maizemeal agar plate cultures. All of the cultures in question had been used in the isolation of species of *Pythium* from diseased portions of seed plants, some having been started with decaying parts of water-lily (*Nymphaea odorata* Ait.) leaves collected near Butternut, Wis., late in July 1935, and others with decaying pieces of stems and roots cut from tomato (*Lycopersicon esculentum* Mill.) plants found wilting in the greenhouse early in December 1935. To each of the cultures had been added, besides, pinches of leaf mold taken from supplies of this material collected during July 1935, partly in deciduous woods near Cumberland, Md., and partly in coniferous woods near Butternut, Wis. As the fungus, wherever observed in its earlier development, always began spreading over the substratum from a deposit of leaf mold, its presence in the cultures must have been attributable to

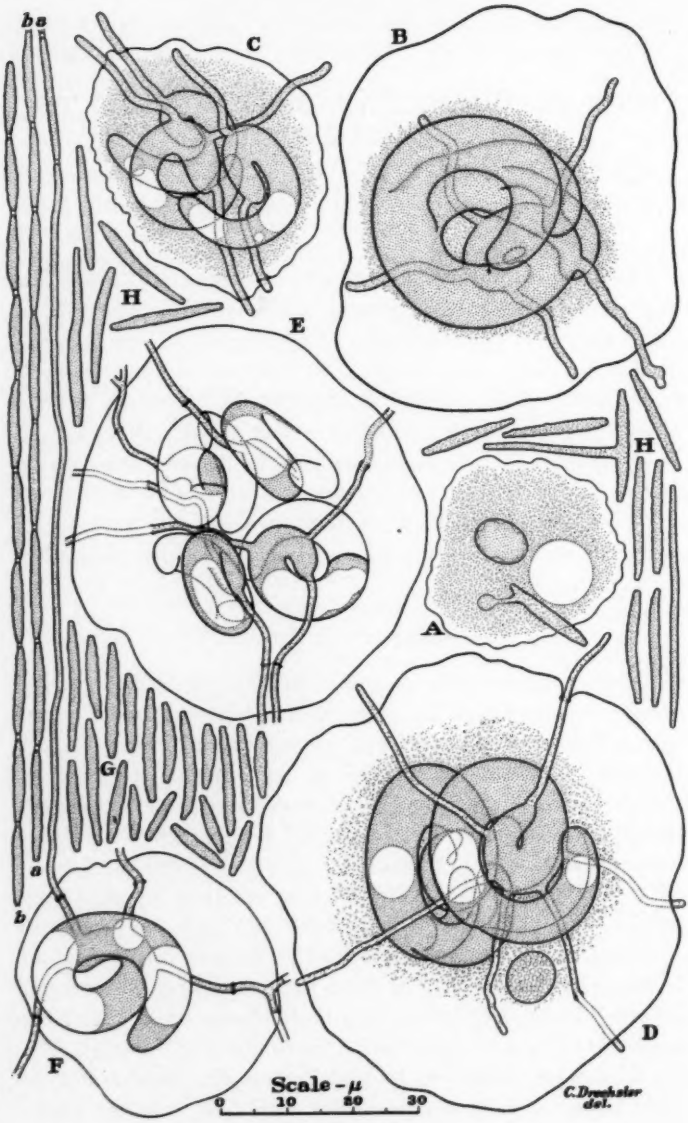

FIG. 1. *Cochlonema odontosperma.*

the forest refuse rather than to the pieces of freshly decaying herbaceous tissue. Whenever encountered it subsisted exclusively as a parasite on what appeared to be a single species of *Amoeba* that, in an approximately rounded shape, measured mostly from 30 to 60 μ in diameter. Although the finely granular protoplasm of the newly infected animals was not unfavorable for microscopic examination, a body of dimensions and structure to encourage interpretation as a nucleus could not usually be made out as clearly as might be desired. An ellipsoidal inclusion discerned in some invaded specimens and revealing a darkish peripheral layer with lumpy thickenings protruding inward (FIG. 1, *A*), appeared the most persuasive among the likely structures that came under observation. Possibly the same structure in a pathological condition may have been represented in a body of comparable size, showing a layer of separate peripheral granules suggestive of chromatin particles, that was seen in some nearly depleted animals (FIG. 1, *D*). With either type of peripheral organization, interpretation was embarrassed by the presence frequently of digestive vacuoles somewhat similar in dimensions, in shape and occasionally even in arrangement of contents; so that for the time being the specific identity of the susceptible animal remains, unfortunately, very uncertain.

Infection of the *Amoeba* is initiated when an adhering conidium thrusts a germ tube through the pellicle and a short distance into the protoplasmic interior. At the tip of the germ tube a globose swelling then puts into appearance (FIG. 1, *A*) and increases in size with the transfer to it of the conidial contents. Completion of the transfer is followed by disarticulation of the globose body, which immediately begins autonomous development, while the empty envelope of conidium and germ tube is promptly expelled. Invasion thus follows the course set forth with more attention to detail in my description of *Endocochlus asteroides* (**1**) and *E. gigas* (**2**). Just as in the two species mentioned, the vegetative body here develops as a thick filament, which, whether simple or dichotomously branched, is coiled in a handsome cochleate spiral of one to two turns. Because in this species an empty conidial membrane has never been seen attached to a convolved thallus, separation from the two congeneric forms I described earlier (**1**)

as *Cochlonema verrucosum* and *C. dolichosporum,* which regularly show such attachment, entails no difficulty.

That the fungus is, indeed, a third member of the genus *Cochlonema,* is plainly revealed in its asexual reproduction. The conidiiferous hyphae are proliferated radially from the outer contour of the spiral body, the one first produced arising close to or often directly from the place of original attachment, and additional ones arising at intervals mostly of 3 to 10 μ in positions successively closer to the growing extremity (FIG. 1, *B–F*). Naturally the number of such hyphae is variable: a poorly developed thallus that has shared the nourishment available in a small animal with several fellows may produce only a single conidiiferous filament (FIG. 1, *E*), whereas five (FIG. 1, *B*), six (FIG. 1, *D*) or even more may originate from relatively massive vegetative bodies. If the host dies submerged in the substratum, the filaments make their way to the surface, where differentiation somewhat gradually becomes evident in slightly increased width, in regularly spaced constrictions, and in perceptible verrucose sculpturing. The conidia resulting from evacuation of the constrictions and delimitation of the separated protoplasts by end walls, are thus distinguished by minutely warty contours (FIG. 1, *F, G, H*). Such sculpturing may be virtually absent, however, in the small admixture of longer and narrower spores resulting from segmentation of the little differentiated proximal portions of the aerial prolongations. Since branching occurs, though, of course, not profusely, in the sporogenous portions of the filaments as well as in the sterile submerged portions (FIG. 1, *F*), branched conidia are occasionally to be found (FIG. 1, *H*).

Sexual reproduction takes place in some quantity usually during the later stages of an epizoötic, when conidia are strewn about in such abundance that plural infections frequently occur with the result that many individual animals come to harbor two or more thalli of the consistently dioecious parasite. In position of origin the zygophoric hyphae are similar to the conidiiferous filaments, which, moreover, in their proximal portions they exceed little in width. After attaining some length, and especially on passing through the pellicle of the host, they usually widen markedly; further elongation with some bending thereupon bringing about

apical union in pairs, followed soon by fusion (FIG. 2, *C*, *a*). Some zygophoric hyphae, to be sure, fail to find an unengaged mate (FIG. 2, *A*, *e*, *f*, *g*; *C*, *e*), and then may continue to grow a little, often with the bizarre erraticness elsewhere familiar in instances of such frustration. Now and again three sexual hyphae, with a fine disregard of propriety, come together in a triple union (FIG. 2, *A*, *d*). In any case, conjugation is followed by the budding forth of a globose excrescence from one of the hyphae, generally at some distance from the union (FIG. 2, *A*, *a–d*). This excrescence, the young zygosporangium, remains smooth until about fully grown, when 20 to 35 warty prominences appear on the spherical surface (FIG. 2, *B*, *a–d*), soon to become modified individually like the crown of a grinding tooth with two, three or four recognizable cusps (FIG. 2, *C*, *b–d*). The decided increase in thickness of wall that takes place during maturation (FIG. 2, *D*, *a–d*; *E*, *a–f*) is no doubt to be attributed to the deposition of a zygospore wall proper, which, at least under the difficult optical conditions brought about by the pronounced sculpturing, appears indistinguishably fused with the zygosporangial envelope.

An epithet composed of two words meaning "tooth" and "seed" respectively, would seem appropriate for the fungus.

Cochlonema odontosperma sp. nov.

Hyphae alitae 4–10 μ crassae, simplices vel semel vel bis dichotomae, semel vel bis spiraliter convolutae. Conidia cylindracea, utrimque attenuata, rarius paulo ramosa, plerumque minute verrucosa, 8–36 μ longa, 1.2–2.0 μ crassa, in catenulas saepe 10–30-sporas digesta. Hyphae zygosporiferae 20–45 μ longae, basi 1–1.5 μ crassae, sursum 3–4 μ crassae, utraque ex alia hypha alita enata. Zygosporangia sphaeroidea, 8–10 μ diam., membrana 20–35 dentibus columellaribus, minute bicornibus vel tricuspidibus vel minute quadrifidis, 1–1.5 μ altis et latis ornata. Zygosporae paulo flavidae, membrana circa 1–1.2 μ crassa cum membrana zygosporangii verisimiliter concreta, quacum conjunctim loculum 6–7 μ diam. circumdat.

Amoebas 30–60 μ latas enecans et consumens, habitat in humo silvarum prope Cumberland, Maryland, et Butternut, Wisconsin.

Vegetative hyphae 4 to 10 μ in diameter, frequently simple or once bifurcate, but only rarely twice bifurcate, coiled beautifully in a spiral of 1 to 2 turns. Conidia cylindrical, tapering at both ends, rarely branched, mostly minutely warted, 8 to 36 μ, mostly 10 to 20 μ (average 16 μ) long, and 1.2 to 2 μ (average 1.6 μ)

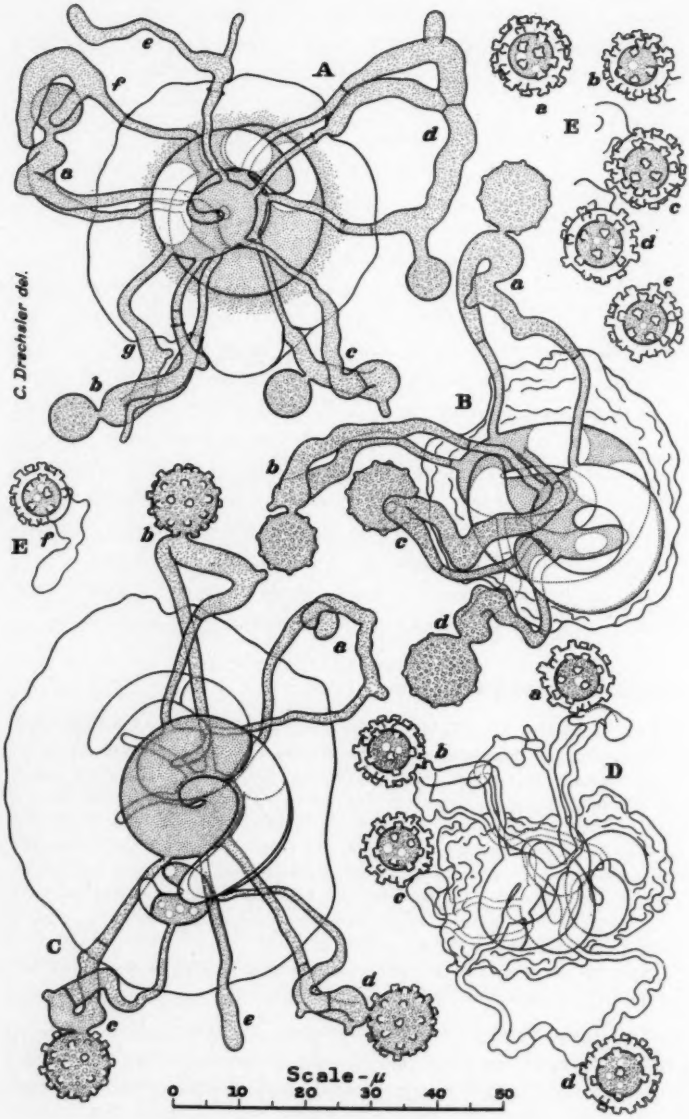

FIG. 2. *Cochlonema odontosperma.*

wide, formed in chains in numbers varying mostly from 10 to 30. Zygophoric hyphae 20 to 45 μ long, 1 to 1.5 μ wide at the base and 3 to 4 μ toward the distal end, each of a conjugating pair arising from a separate assimilative hypha. Zygosporangium subspherical, 8 to 10 μ in diameter, its membrane ornamented with 20 to 35 columnar toothlike protuberances, mostly 1 to 1.5 μ in height and in width, and bearing 2, 3 or 4 minute cusps. Zygospores somewhat yellowish, having a proper wall perhaps about 1 to 1.2 μ in thickness, that appears indistinguishably adnate to the zygosporangial membrane, together with which it surrounds a locule 6–7 μ in diameter.

Occurring in leaf mold, infecting and consuming *Amoebae* 30 to 60 μ in diameter, near Cumberland, Md., and Butternut, Wis.

COCHLONEMA MEGASPIREMA

Of the three species of *Endocochlus* hitherto described, *E. gigas* is easily the most impressive, surpassing its two known congeners in the bulk and elaborately helicoid convolution of its thallus, as well as in the abundance of sexual and asexual reproductive apparatus produced therefrom. This luxuriance, as was pointed out in the original description, is made possible by the size and substantial composition of the host animal, *Amoeba terricola* Greef (*sensu strictiore*), a rhizopod, which, if not strictly of rare occurrence in old agar plate cultures, certainly does not seem to develop there in considerable numbers as frequently as might be expected from its reputedly general distribution in the soil. The animal, easily identified by the characteristic structure of its large nucleus (FIG. 3 *A*, *n*) was observed in quantity in some plate cultures as one of the numerous organisms superimposed on *Pythium* mycelia that had promptly grown out from decaying pieces of leaves of the white water lily collected near Butternut, Wis., late in July 1935. To a number of cultures thus infested were added some pinches of partly decayed tomato leaves picked up from the ground in a greenhouse near Beltsville, Md. In these cultures, two weeks later, the entire population of the rhizopod, consisting perhaps of a hundred individuals, was being exterminated by an endoparasite of most extraordinary appearance.

Development of the parasite begins, as could be determined from observations on a few favorable incipient infections, much like

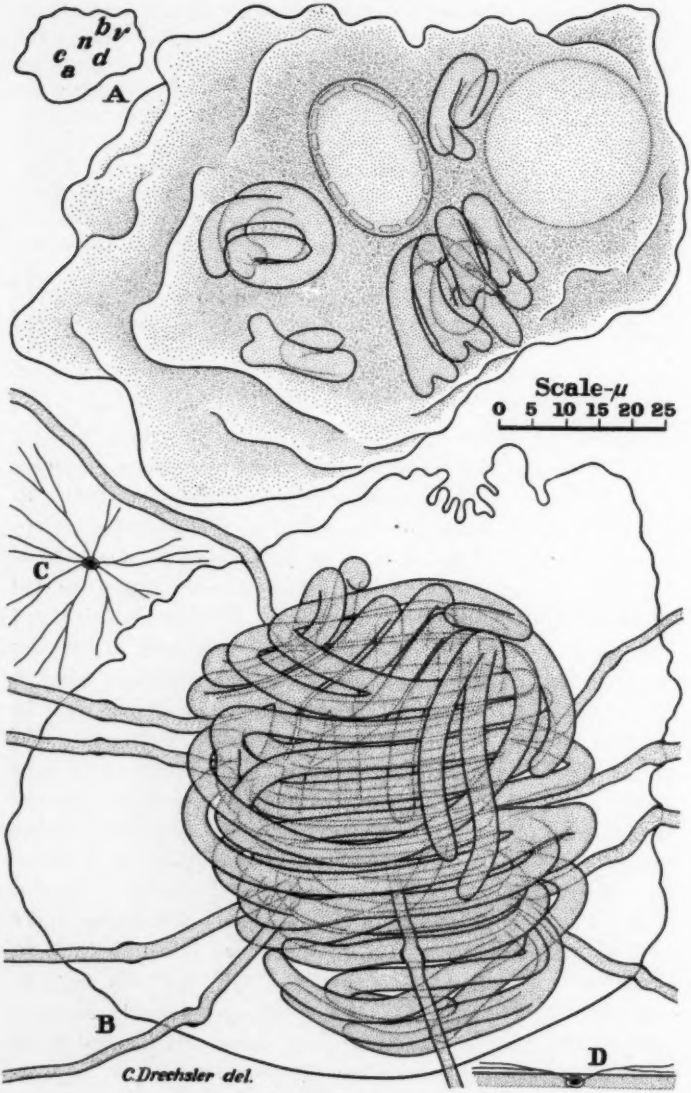

FIG. 3. *Cochlonema megaspirema.*

development of *Endocochlus asteroides, E. brachysporus* Drechsl., *E. gigas* and *Cochlonema odontosperma:* a germ tube, thrust into the animal by an adhering conidium, giving rise to an apical globose body, which, after receiving all the conidial contents, becomes detached to proceed with autonomous growth. On attaining a length of approximately 10 μ the young thallus, like the thalli of other endoparasitic members of the Zoöpagaceae, curves abruptly to commence the spiral windings that thereafter are continued until the protoplasmic materials of the host are finally exhausted. However, while in the other endoparasitic forms a marked widening of the thallus is evident at the first turn if not before, in the present species the filament maintains an approximately constant width little greater than that of the newly disarticulated globular body. The much greater length of filament thus made necessary contributes to more frequent branching and to far more elaborate coiling; so that vegetative bodies with one to two bifurcations and as many turns (FIG. 3, *A, a, b, c*) represent very early stages of development, and even a thallus with two successive turns and three successive dichotomies (FIG. 3, *A, d*) may be still relatively immature. When a single thallus has completed appropriation of the contents of a large animal (FIG. 3, *B*), it appears as an intricate mycelial coil too thick to be seen through, though the elements exposed to view in the upper aspect and at the periphery indicate sufficiently that the number of successive dichotomies certainly can not be less than four or five. Of course, much less impressive development results when a number of thalli share the contents of a relatively small host animal (FIG. 4, *A*).

Asexual reproduction always takes place in quantity, beginning as soon as the host animal has been brought to a stop, usually at some depth in the agar substratum. Even while the last protoplasmic remnants of the *Amoeba* are being appropriated, a number of filaments are put forth from the coiled thallus. These perforate the substantial pellicle of the host after the manner of an appressorium, and then continue to elongate toward the surface of the substratum, their contours becoming in increasing measure irregularly and minutely warty (FIG. 4, *B, a–c*). After emergence into the air, elongation is continued in a nearly horizontal direction (FIG. 4, *B, c–d*) until a length usually of several milli-

meters has been reached (FIG. 3, *C, D*). Sporulation thereupon takes place as in other catenulate members of the family, that is, through the withdrawal of contents from the short, slightly constricted isthmi perceptible at rather regular intervals in the aerial prolongations (FIG. 4, *B, e–g*), followed by the laying down of septa at both ends of the protoplasts thus separated (FIG. 4, *B, d–k*). Frequently a proximal part of an aerial prolongation is not immediately converted into conidia, instead growing out anew from below the lowermost conidium to give rise to a filament that in due course similarly yields a chain of spores. Repetition of the same process several times in each of the hyphal outgrowths coming from the thallus, in the end brings about a somewhat arachnoid display of catenated conidia with a total bulk seemingly far in excess of what might be expected from the volume of the underlying vegetative coil, even though this volume is in many instances obviously very considerable.

In general appearance the conidia (FIG. 4, *C, a-q*) bear most resemblance to those of *Zoopage phanera* Drechsl., though usually they are somewhat shorter and noticeably more coarsely sculptured. Their slightly tapering and bluntly rounded or truncate ends recall *Z. nematospora* Drechsl., and, as in the latter species, the little constricted empty connections between the members of a chain reveal the mode of sporulation with satisfying clearness.

Sexual reproduction took place in only one of the cultures, and there only very sparingly. The process shows similarity to that set forth in my description of *Bdellospora helicoides,* the distally widened zygophoric hyphae, of which each in a pair arises from a separate thallus, becoming intervolved in a few helicoid turns. One of the conjugating hyphae is usually somewhat in advance of its mate, and thus alone provides the forefront of the intervolved helices from which the zygosporangium buds forth as a sessile globose excrescence (FIG. 4, *D, a-d*). The fully grown zygosporangium, like the homologous structure of *B. helicoides* is ornamented with warty protuberances. In spite of diligent search it was not possible to find mature sexual apparatus whose connection with the fungus under consideration was attested by positional relationship to a pellicle recognizable as pertaining to *Amoeba terricola.* A single cluster of mature zygospores encountered after

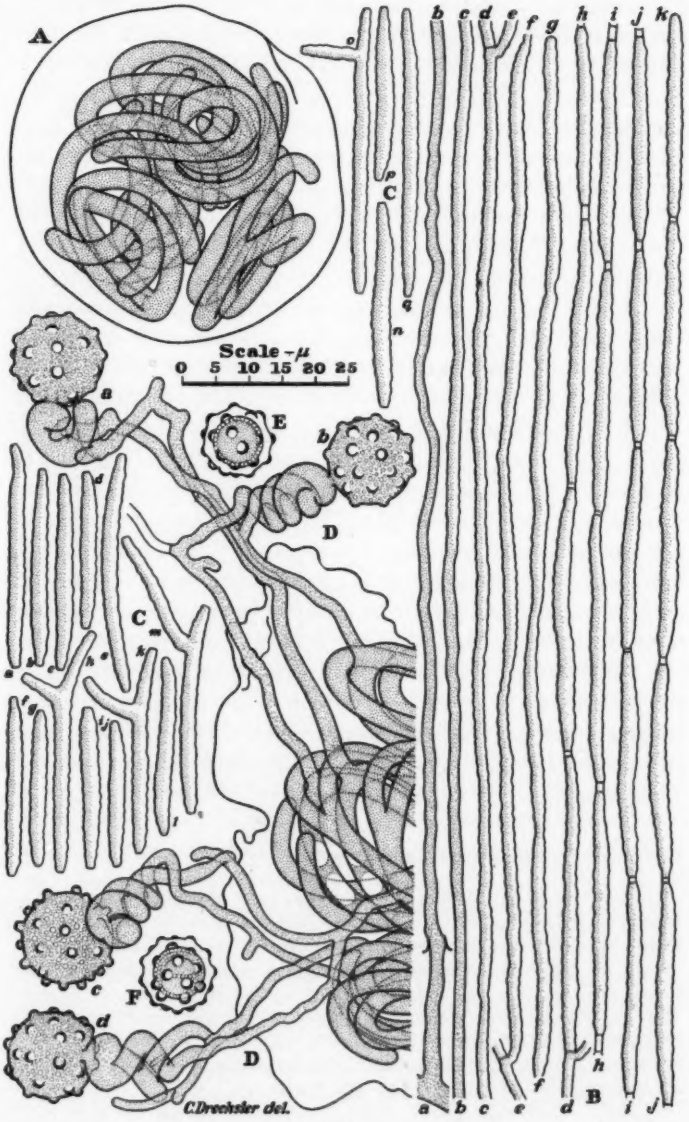

FIG. 4. *Cochlonema megaspirema.*

the disappearance of the pellicle had made their identification uncertain, probably belonged to the parasite even though their dimensions were somewhat smaller than might have been expected They showed a thick zygospore wall, which, fused indistinguishably with the warty zygosporangial envelope, inclosed a locule containing a parietal layer of coarse granules and a large central reserve globule (FIG. 4, *E, F*).

While I was a student in the Cryptogamic Laboratories of Harvard University 20 years ago, the late Professor Thaxter took occasion to show me under his microscope a very well developed thallus either of the present fungus or of a fungus exceedingly similar to it. In reply to an inquiry as to the nature of so amazing a structure he remarked that " Its name is enigma." The meager information I retained of the incident, on which, nevertheless, Dr. W. H. Weston and Dr. D. H. Linder acted with much kindness, proved insufficient to locate the preparation in the collections left at Harvard by the distinguished mycologist. It seems probable that the specimen shown me in 1916 appeared adventitiously in a mount of some other fungus, perhaps when the living material had already been discarded, giving no opportunity for relating the curious thallus to a spore form, or even for identifying the substantial enveloping membrane as the pellicle of a parasitized terricolous *Amoeba*. As Thaxter demonstrably used an unpublished term " Aenigma " in a sense quite alien to any possible application suggested in the oral remark quoted above, a name made up from two words meaning " large " and " coil " respectively, is proposed for the fungus.

Cochlonema megaspirema sp. nov.

Hyphae alitae 2.5–4.5 diam., saepe, praecipue ubicumque solitariae in animalibus magnis luxuriantes, longae, quater vel quinquies repetite dichotomae, in spiram miram circumplicantes. Conidia verrucosa, anguste cylindracea vel filiformia, interdum ramosa, utrimque paulo attenuata, 20–45 μ longa, 1.6–3 μ crassa, in catenulas longas, simplices vel furcatas digesta. Hyphae zygosporiferae circa 50–80 μ longae, deorsum interdum aliquam ramosae et circa 2 μ crassae, sursum circa 4 μ crassae et binae inter se bis vel quater spiraliter circumplicantes. Zygosporangia 10–15 μ diam., flavida, 20–30 verrucis .8–1.5 μ altis 1.5–2 μ latis ornata.

Amoebam terricolam (sensu strictiore) enecans, habitat in foliis semisepultis putrescentibus *Lycopersici esculenti*, prope Beltsville, Maryland.

Vegetative hyphae 2.5 to 4.5 μ (average 3.6 μ) in width, often and more especially on developing singly in large animals, conspicuously long, repeatedly bifurcate 4 or 5 times and wound rather compactly in coils of numerous individual turns. Conidia distinctly warty, narrowly cylindrical or filiform or sometimes branched, slightly tapering toward the ends, 20 to 45 μ (average 31 μ) long and 1.6 to 3 μ (average 2.3 μ) wide, produced in numbers up to 50 or more in long, simple or branched chains, wherein they are separated from one another by evacuated portions of filament usually about 1.3 μ wide and .5 to 3 μ (mostly about 1 μ) long. Zygophoric hyphae often about 50 to 80 μ long, proximally sometimes branched and measuring about 2 in width, distally widening to about 4 μ and winding about one another in pairs 2 to 4 times, each of a pair originating from a separate thallus. Zygosporangium sessile on the conjugating filament providing the forefront of the spiral, 10 to 15 μ in diameter, nearly colorless or faintly yellowish, its wall ornamented with 20 to 30 warty protuberances .8 to 1.5 μ high and 1.5 to 2 μ wide.

Occurring as a destructive parasite of *Amoeba terricola* (in a more restricted sense) in partly buried decaying leaves of *Lycopersicon esculentum* near Beltsville, Md.

ZOOPAGE TRYPHERA

A member of the Zoopagaceae subsisting on testaceous rhizopods appeared in several old maizemeal agar plate cultures to which had been added a few pinches of leaf mold collected in deciduous woods near Butternut, Wis., late in July 1935. Mixed with other forms of microscopic life, *Geococcus vulgaris* Francé (**4**) had become rather uniformly, even if somewhat sparingly, distributed on the surface of the agar substratum. Marked concentration of these animals, often in conspicuous linear arrangement and always in restricted areas bordering on one or another of the deposits of leaf mold, revealed the extension of predacious mycelia from the decaying rubbish. As conidial apparatus of the *Fusarium*-like hyphomycete I described earlier (**3**) under the binomial *Dactylella passalopaga* was nowhere to be seen, a closer examination was made, which brought to light, instead, a delicate species of *Zoopage* much more commonplace in its predacious habit.

Capture was effected manifestly through mere adhesion of the animals on the narrow mycelial filaments (FIG. 5, *A; B, a-f; C, a*).

If, as may be presumed, yellow adhesive material similar to that produced by related fungi was operative here, its recognition was rendered difficult in the presence of a yellowish plug, often of considerable bulk, in the mouth of each captive, where it had been secreted apparently in an effort to resist invasion. In each instance, nevertheless, a very delicate branch from the axial hypha traversed the plug, and after growing a short distance into the interior of the animal, widened and branched dichotomously a number of times to give rise to a well differentiated haustorium through which the protoplasmic contents were gradually appropriated.

Asexual reproduction of the fungus (FIG. 5, *C, b; D, a-d*) was rather scanty owing probably in part to the meager supply of *Geococcus vulgaris* available in the cultures. In dry mounts the conidia (FIG. 5, *E*) could be seen to be covered with minute warts, which, however, for the most part became invisible in moist preparations. As in some other catenulate members of the family, the 2 or 3 proximal conidia in the better developed chains were usually longer, narrower, and less regularly sculptured than those borne in more distal positions.

Because of its relatively small dimensions and a generally frail appearance throughout, the species may be aptly described under a name meaning " delicate."

Zoopage tryphera sp. nov.

Mycelium ramosum, sparsum; hyphis hyalinis, .7–1.3 μ crassis; haustoriis pedicellatis, pedicello 2–7 μ longo, .5–.7 μ crasso, saepe sursum incrassato, aliquot ramulos repetite dichotomos .8–2.5 μ crassos sursum attenuatos ferente. Conidia minute verrucosa, elongato-fusoidea, 6–22 μ longa, 1.2–2.2 μ crassa, in catenulis plus minusve erectis ex apice sterigmatum brevium sed saepe ramosorum oriunda, in quaque catenula saepius quina usque quina dena. Zygosporae ignotae.

Geococcum vulgarem capiens et consumens, habitat in humo silvarum prope Butternut, Wisconsin.

Mycelium branched, sparse; hyphae hyaline, .7 to 1.3 μ wide; haustoria pedicellate, the pedicel mostly 2 to 7 μ long and .5 to .7 μ wide, often broadening markedly before bifurcating into branches up to 2.5 μ wide, which in turn bifurcate once, twice or three times, usually with progressive attenuation of the elements successively formed to a width of .8 to 1.3 μ at the tips. Conidia very minutely

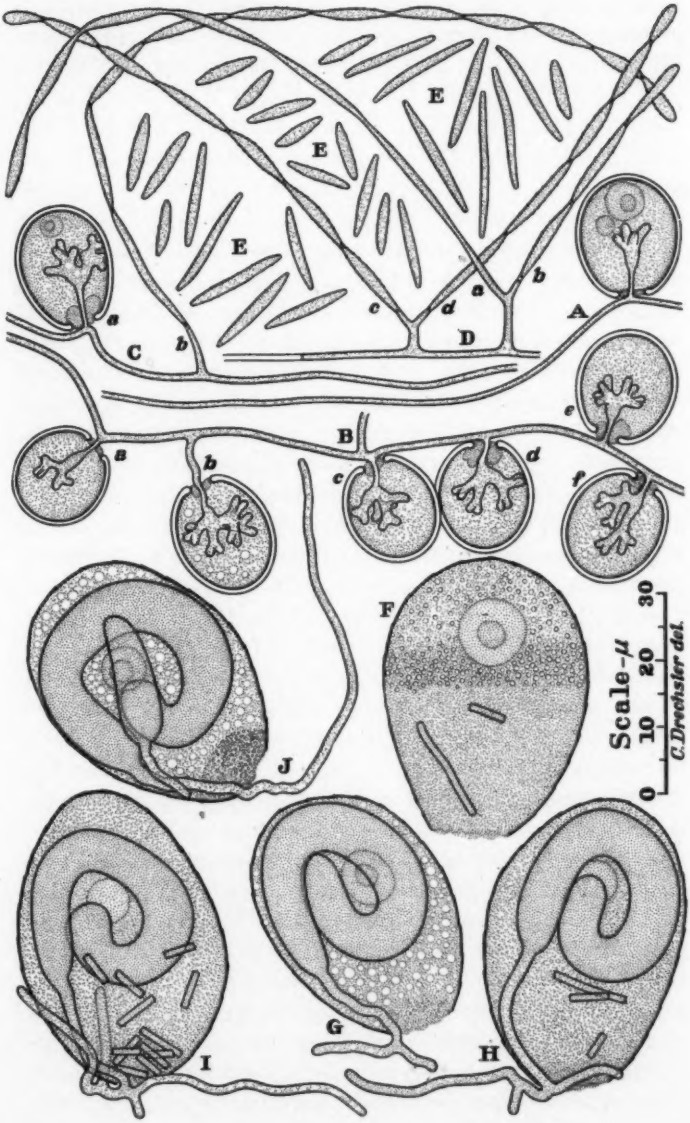

FIG. 5. *A–E, Zoopage tryphera; F–J, Cochlonema cylindricum.*

warted, mostly elongated spindle-shaped, 6 to 22 μ (average 13 μ) in length, 1.2 to 2.2 μ (average 1.7 μ) in width, produced in numbers usually from 5 to 15 in more or less erect chains arising from short yet sometimes branched sterigmata. Zygospores unknown.

Occurring in leaf mold, capturing and consuming *Geococcus vulgaris,* near Butternut, Wis.

COCHLONEMA CYLINDRICUM

A morphologically very distinctive species of *Cochlonema* made its appearance several times in aging maizemeal agar plate cultures originally planted with largish pieces of decaying stem and root tissue cut from tomato plants found wilting in the greenhouse early in December 1935, evidently as the result of root-rot and foot-rot injury. The fungus in question subsisted parasitically on a testaceous rhizopod of the genus *Euglypha.* A somewhat more regularly ovoid contour, and aperture scales neither thickened nor incurved at their tips, readily distinguished the host animal from the congeneric *E. laevis* Perty, cited earlier (**3**) as prey of *Dactylella passalopaga.* In the dimensions of its glabrous test and of its scales, as well as in the size and position of its nucleus, the rhizopod corresponded well to the description of *E. denticulata* Brown as given by Wailes (**5**), and is therefore referred to that species. It first attracted my attention by feeding on the oöspores of *Pythium ultimum* Trow scattered abundantly through the agar substratum; in each case applying its mouth to the oöspore wall, digesting a hole through the wall in the area thus circumscribed, and then drawing out the degenerating granular contents, as if by suction. Unlike *Geococcus vulgaris,* which attacks oöspores in precisely the same manner, the animal multiplied rather slowly, its numbers in any of the 4-inch Petri dishes probably not exceeding 100 after a period of 35 days, when destruction by the parasite first became noticeable. Complete extermination followed in less than a week.

Infection of the individual rhizopod results when a conidium of the fungus is ingested (FIG. 5, *F*) that on germination gives rise to a thick spiral thallus (FIG. 5, *G–J*) very similar to the thalli of *Cochlonema verrucosum* and *C. dolichosporum.* After the animal's contents have been partly assimilated, the empty spore

membrane frequently becomes visible as a cylindrical appendage attached endwise to the thallus (FIG. 6, *A–C*) indicating that in this species germination is approximately terminal rather than lateral. Attachment, moreover, is at the end of the swollen filament that occupies not the central but the more outward position in the spiral; the cochleate shape here manifestly coming about through introversion of the growing distal portion, rather than through circumvolution as in congeneric forms. This departure from the habit of growth usual in the genus is, perhaps, to be related to the presence of the enveloping rounded test, and to freedom from the continuous indiscriminate rolling entailed in the movement of pelliculate *Amoebae*. Frequently two thalli develop in an infected animal (FIG. 5, *J*; 6, *A*), and even three have been observed in a few instances. Though additional conidia are often ingested (FIG. 5, *H, I*), these for some reason fail to germinate, long remaining visible in the protoplasm of the rhizopod without undergoing any evident change.

Asexual reproduction of the fungus begins usually before the conclusion of vegetative development, being initiated apparently as soon as the host animal has become too feeble to move about, though generally a considerable portion of its substance may still await appropriation. A narrow hypha arising from the older extremity of the thallus makes its way to the mouth of the rhizopod, from which it emerges often with some branching and an abrupt change in direction (FIG. 5, *G–J*). Penetration into the substratum is usually not extensive, all later growth being in most instances wholly aerial (FIG. 6, *A*). In places where several infected animals have been halted fairly close together, the filaments proceeding from them often become more or less entangled with one another in rangy curling loops, to provide a woolly display much more profuse than might be expected from the volume of the subjacent thalli. As each of the filaments attains definitive length it becomes converted into a chain of sharply truncate cylindrical spores (FIG. 6, *B–E*). A second filament may grow out from below the proximal member of the chain to give rise to a second conidial chain. Through repetition of such development, additional chains are produced until the progressive evacuation of the underlying thallus has been completed, leaving only a num-

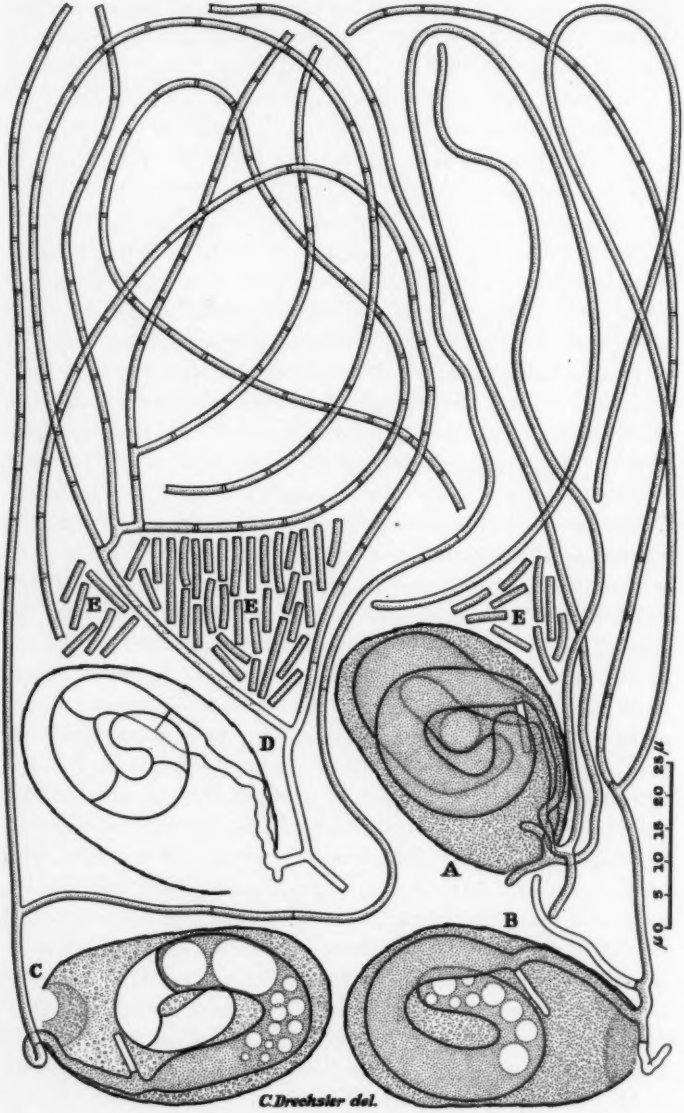

FIG. 6. *Cochlonema cylindricum.*

ber of successive cross-wall in the empty cochleate envelope (FIG. 6, *D*).

Cochlonema cylindricum sp. nov.

Hyphae alitae 5–8.5 diam., simplices, semel vel bis introrsum spiraliter convolutae. Conidia hyalina, cylindracea, utrimque abrupte truncata, recta vel leniter curva, 4–17 μ longa, 1.1–1.3 μ crassa, in catenulas longas, simplices vel basi paulo ramosas, flexuosas, saepe inter se aliquanto intricatas connexa. Zygosporae ignotae.

Euglypham denticulatam enecans habitat in radicibus putrescentibus *Lycopersici esculenti* prope Beltsville, Maryland.

Vegetative hyphae mostly 5 to 8.5 μ in diameter, spirally convoluted in a circinate coil of 1 to 2 turns. Conidia hyaline, cylindrical, abruptly truncate at both ends, straight or slightly curved, 4 to 17 μ, mostly 5 to 10 μ (average 7.2 μ) long and 1.1 to 1.3 μ wide; produced in large numbers in long, flexuous, simple or basally branched, sometimes distally intertwined chains, wherein they are separated by evacuated but not constricted portions of filament mostly about .3 μ long. Zygospores unknown.

Parasitic on *Euglypha denticulata* and occurring in decaying roots of *Lycopersicon esculentum* near Beltsville, Md.

BUREAU OF PLANT INDUSTRY,
U. S. HORTICULTURAL FIELD STATION,
BELTSVILLE, MARYLAND

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EXPLANATION OF FIGURES

Fig. 1. *Cochlonema odontosperma;* drawn with the aid of a camera lucida at a uniform magnification; × 1000 throughout. *A*, A small susceptible *Amoeba* being infected by a germinating conidium; close to the contractile vacuole is shown a body believed to be the host nucleus. *B*, A twice bifurcate thallus that has attained its rather unusually large dimensions by de-

veloping alone in an animal of good size; from the outer profile of its older portion have been produced five young conidiiferous filaments. *C*, Three small thalli putting forth from the relatively small animal in which they have developed, one, two and three conidiiferous filaments respectively. *D*, Two thalli, each once bifurcate, which have nearly depleted their host; one of the thalli putting forth six conidiiferous filaments. *E*, Four simple thalli whose contents have been largely used up in asexual reproduction, the resulting conidial chains not being shown from lack of space. *F*, A simple thallus partly depleted of contents in asexual reproduction, one of the shorter conidial chains being shown in sections; *a* and *b* representing corresponding points in these sections. *G*, A random assortment of conidia. *H*, An assortment of conidia, including mostly branched, filamentous, and relatively large specimens.

Fig. 2. *Cochlonema odontosperma;* drawn with the aid of a camera lucida at a uniform magnification; × 1000 throughout. *A*, Sexual hyphae from two thalli in an exhausted animal, three pairs, *a*, *b* and *c*, having conjugated to form each a young zygosporangium; *d*, a somewhat unusual triple union; *e*, *f*, *g*, zygophoric hyphae that have failed to make contact with a free mate. *B*, Two thalli that have given rise to four pairs of sexual hyphae, *a-d;* each pair having produced a fully grown zygosporangium showing an early stage in the formation of protuberances. *C*, Three thalli from which have come a pair of young sexual hyphae distally in contact with one another, *a;* three pairs of older sexual hyphae, *b*, *c*, *d*, that have each produced a zygosporangium with fully grown protuberances; and a young unmated sexual hypha, *e*. *D*, Four mature zygospores, *a-d*, together with the empty sexual hyphae, the evacuated vegetative thalli and the collapsed pellicle. *E*, Mature zygospores, *a-f*, showing variation in size and sculpturing.

Fig. 3. *Cochlonema megaspirema*. *A*, Specimen of *Amoeba terricola* in active movement, though infected with four young vegetative thalli, *a-d; n*, nucleus; *v*, contractile vacuole; drawn with aid of camera lucida, × 1000. *B*, A well developed vegetative thallus with the proximal portions of 10 conidiiferous filaments thrust through the substantial pellicle of a depleted large specimen of *A. terricola;* drawn with aid of camera lucida, × 1000. *C*, Sketch showing radial arrangement of conidiiferous filaments about the underlying thallus. *D*, Sketch showing course of conidiiferous filaments through substratum and recumbent posture of aerial prolongations.

Fig. 4. *Cochlonema megaspirema;* drawn with the aid of a camera lucida at a uniform magnification; × 1000. *A*, Three relatively small thalli within the pellicle of a wholly depleted specimen of *Amoeba terricola*. *B*, Conidiiferous filament drawn in sections, the contiguity of which is indicated by the letters *a-k; a-c*, submerged proximal portion; *c-d*, little differentiated proximal portion of aerial prolongation; *d-h-k*, chain of spores resulting from conversion of the well differentiated distal portion of the filament as at first produced; *e-g*, differentiated branch growing from below the basal member of the mature conidial chain. *C*, A random assortment of conidia, *a-q*, showing variation in size and shape. *D*, Portions of three thalli with four pairs of distally intervolved sexual hyphae that have produced four fully grown zygosporangia, *a-d*, respectively. *E*, *F*, Zygosporangia with mature zygospores, considered as probably belonging to the fungus.

Fig. 5. Drawn with the aid of a camera lucida at a uniform magnification; × 1000 throughout.

A–E, Zoopage tryphera: A, Portion of hypha with an incompletely developed haustorium inside of a captured specimen of *Geococcus vulgaris. B,* Portion of hypha with six captured specimens of *G. vulgaris, a-e,* each of which, with the exception possibly of *a,* reveals a fully developed haustorium in its interior. *C,* Portion of hypha showing a fully developed haustorium in the captured specimen of *G. vulgaris, a;* and also a chain of conidia on the sterigma, *b. D,* Portion of hypha with two branched sterigmata: one bearing an immature sporogenous filament, *a,* in addition to the mature conidial chain, *b;* the other bearing two mature conidial chains, *c* and *d. E,* Conidia, showing variation in size and shape.

F–J, Cochlonema cylindricum: F, Two conidia shortly after ingestion by a specimen of *Euglypha denticulata. G–I,* Specimens of *E. denticulata* each containing a single thallus, which has established contact with the substratum by means of a slightly branched filamentous outgrowth; in *H* and *I* are shown also inside of the animal some ingested conidia that betray no sign of germination. *J,* Specimen of *E. denticulata* with two thalli, the larger having thrust a hyphal outgrowth through the mouth of the host.

Fig. 6. *Cochlonema cylindricum;* drawn with the aid of a camera lucida at a uniform magnification; × 1000. *A,* Specimen of *Euglypha denticulata* containing two thalli, each of which has produced a growing aerial filament. *B,* Specimen of *E. denticulata* containing a thallus that after giving rise to a chain of conidia is supplying material for the continued elongation of a sporogenous branch proliferated from below the proximal member of the chain. *C,* Specimen of *E. denticulata* containing a thallus that has become partly evacuated in the production of two long conidial chains; these chains being shown only in part from lack of space. *D,* Specimen of *E. denticulata* reduced to an empty test in supplying nourishment to a thallus that now is represented only by an empty membrane; four septa were laid down in the membrane during the progressive evacuation that resulted in six long conidial chains shown only in small part from lack of space. *E,* Conidia, showing variation in size and shape.

NEW SPECIES OF HYPHOMYCETES

DAVID R. SUMSTINE

Rhinotrichum Noblesiae sp. nov.

Stratum very thin, effused, floccose to definite crust-like layer, brownish-yellow, raw sienna-antique brown in Ridgeway's Color Standards and Nomenclature, with white patches and streaks scattered over the surface; hyphae hyaline, septate, branched; sporophores long, branched, septate, attenuate upwards, with upper division bearing spicules; spores ovoid to ellipsoid, hyaline to yellow by transmitted light, with granular contents, variable in size, median size 10–12 × 14–16 μ.

On coniferous boards, Arctic Ice-House, Winnipeg, Manitoba, September 5, 1935.

This species resembles *Rhinotrichum tenerum* Sumstine. It may be separated from that species by the difference in color, shape and size of the spores, and the lack of H-shaped formations in the hyphae.

The specimens were contributed by Dr. Irene Mounce and Dr. M. K. Nobles of the Dominion Experimental Farm, Ottawa, Canada.

Part of the original collection is deposited in the herbarium of the Carnegie Museum, Pittsburgh, and part in the herbarium of the Dominion Experimental Farm.

Streptothrix Mounceae sp, nov.

Fructification composed of small tufts, 1 mm. or less across, sometimes confluent, brown, near raw umber in Ridgeway's Color Standards and Nomenclature; mycelium hyaline, branched 2 μ in diameter; sporophores erect, repeatedly branched, branches short or at times long, tortuous (corkscrew); spores borne at end of the branches singly or doubly, hyaline at first, then brown colored, ovoid to ellipsoid, one-guttulate, 4–5 × 6–8 μ.

On bark of dead twig, Kenora, Ontario, September 25, 1932.

This species is related to *Streptothrix fusca* Corda. It differs from that species in color and in smaller one-guttulate spores.

The specimen was collected by G. R. Bisby and communicated by Dr. Irene Mounce and Dr. M. K. Nobles, of the Dominion Experimental Farm, Ottawa, Canada.

Part of the original collection is deposited in the herbarium of the Carnegie Museum, Pittsburgh, and part in the herbarium of the Dominion Experimental Farm, Ottawa, Canada.

CARNEGIE MUSEUM,
PITTSBURGH, PA.

LIME-LOVING MOLDS FROM AUSTRALIAN SANDS

CHARLES L. PORTER AND GEORGE ZEBROWSKI

(WITH 1 FIGURE)

The organisms described in this paper were discovered by Mr. Zebrowski while making an examination of sand obtained from Ward's Natural Science Establishment of Rochester, New York. Since this first discovery, various lots of calcareous sands have been placed at our disposal by Dr. J. D. Corrington and Dr. F. H. Ward, both officials of the Ward Establishment. The authors take this opportunity to thank these men for their generous co-operation. These organisms have been found in sands collected from Australia, China, Africa, Texas, North Carolina and the West Indies. They apparently have a world-wide distribution.

The most striking peculiarity concerning these organisms is the fact that they are not indicated merely by their imprints on the surfaces of shells, but they actually penetrate the calcareous substance, producing in many cases an abundant, tangled network of hyphae among which are numerous sporangia, frequently containing spores. This lime-loving trait seems to be common to all forms here listed. Most of these forms were found on fragments of molluscan shells, although they were also encountered on shells of *Foraminifera, Ostracoda,* and even within the spicules of calcareous sponges. As such habitats might suggest all the forms were microscopic. Sands bearing these organisms were also rich in the remains of *Foraminifera, Ostracoda,* fish bones and scales, and sponge spicules. Representative genera of *Foraminifera* included species of *Lagena, Globigerina, Spiroloculina, Textularia* and *Nodosaria.* Since these species range from the Cambrian to the present, the organisms here described as associated with them may or may not be existing at the present time. Remarkably well preserved specimens which are quite common in the sands argue for a recent origin. On the other hand, the fact

that they are embedded in a calcareous matrix may account sufficiently for the excellent state of preservation.

Most of the specimens which are the subject of this paper were exhibited at the 1934 meeting of the Mycological Society at Pittsburgh. Many suggestions were made as to the probable nature of these organisms. It was suggested that algae, tests of *Protozoa* or the holdfasts of *Bryozoa* might be included among the specimens shown. All these suggestions have been carefully considered and a critical reexamination has been made of all the specimens in our collection. Comparisons were made with various incrustations that may be found on molluscan shells, with egg cases of leeches, with cysts of Cercariae of fresh-water snails, with tests of several protozoa (*Radiolaria, Helizoa, Lobosa,* etc.). No specimens were found in these inquiries that could be compared to the organisms here described. In our investigation we have excluded all forms occurring on the surface of shells and have considered only those actually imbedded in the shell. The writers were unable to find any described species of protozoa or other invertebrates which could or do produce microscopic tunnels in the hard lime layers of the shells, spicules, etc., which were found.

The large number of specimens discovered in some of the species, where the characteristics were always constant, preclude, in these forms at least, the possibility that our descriptions are based on artifacts, cracks or checks in the shells, or upon other errors in observation. Although we have a large number of described forms there are many others which were omitted either because they were generalized forms upon which no description could be based or because they might be the remains of some of the invertebrates above mentioned. There are, of course, no absolute criteria by which we can state definitely that some of the described species are not primitive algae rather than molds. There seems to be some evidence that we have here some border-line groups which partake of both fungal and algal characteristics. There are also specimens which suggest that these lower fungi may have myxomycetous origin, as transitional forms showing traits common to both were frequently encountered. It is hoped to discuss these interesting relationships in a forthcoming paper.

The unusual preservation and clarity of details makes it possible to work out the life-histories of a number of species of these fungi. A structure, common to most forms, is the presence of

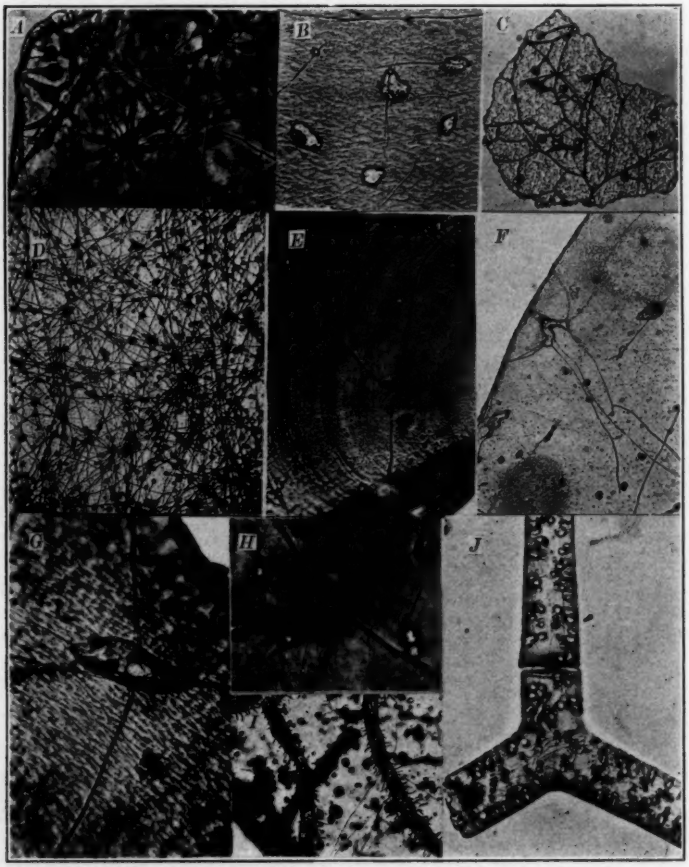


FIG. 1.

pear-shaped or globular sporangia imbedded in the shell substance and opening to the exterior by excretory pores. Usually there is a single pore but in some species there may be several. In many of the species the sporangia are multiple; that is, lateral pouches

or outgrowths arise from the primary sporangium. It is in these, rather than in the primary sporangia, that spores seem to be produced. In some forms the lateral sporangia are produced in linear series presenting somewhat the appearance of a row of gourds with long necks, arranged end to end. In other forms the secondary sporangia are so arranged that the entire sporangial complex resembles the fingers of a hand, or the rays of a star. In a thick shell the lateral sporangia may be symmetrically arranged in the form of star rays. When the shell is very thin, the primary and secondary sporangia may all lie in a single plane simulating a hand with outstretched fingers. Distributive hyphae are also characteristic of most of these molds. These hyphae arise from the walls of either the primary or secondary sporangia and are variable in thickness, but fairly constant for a given species. Usually they are single and unbranched and ramify through the shell for long distances. Eventually at their ends, or subterminal in position, young sporangia are produced which upon developing to maturity produce in turn lateral sporangia and new distributive hyphae. In this manner these molds form a colony of intermixed hyphae which grow from a sporangium. There are frequently other shorter hyphae which are apparently vegetative in nature. In a few forms these nutritive hyphae are arranged in a radial manner at the surface of the shell, the sporangial opening being approximately in the center. In most cases, however, these hyphae as the former, are imbedded within the shell substance and present a rhizoidal appearance. In some species distributive hyphae were observed to terminate in a pair of comma-shaped bifurcations, one of which was larger than the other. These occurred where normally sacs were found on other hyphae and may have sexual significance.

A few forms in which hyphae are entirely lacking resemble a plasmodium with numerous processes and is covered with spines, hairs, or denticles. In such forms the entire thallus apparently functions as a single or multiple sporangium.

The multiple sporangial forms abstrict portions of the protoplasm in the lateral processes, in which the entire protoplasm undergoes transformation into spores. Usually, also, such abstricted portions of the thallus develop their own excretory pores

by which the spores are discharged. As these spores ripen, other areas of the thallus are abstricted, thus gradually transforming the protoplasm of the entire thallus into spores. Usually the apertures are about the size of the spores. However, in some cases the apertures are much smaller than the spores contained within the sporangium, which would suggest that the spores would have to be amoeboid to squeeze their way through. In these latter instances the spores are irregular in outline suggesting strongly amoeboid tendencies. Where, however, the spores are walled and possess regular outlines, the excretory apertures approximate them in size. In color, the spores are usually some shade of yellow or brown, when mature.

It was found possible to isolate hyphae and spores from the matrix by the dissolving action of hydrochloric acid. In some of the coarse specimens so treated, spore-developing areas could frequently be observed along the course of single hypha. The more developed of these areas were apparently cut off from the remaining hyphae by septa. An aggregation of these spores, varying from few to many, would lie surrounded on all sides by an undifferentiated brittle matrix, which crumbled when touched. These spores were of the same greenish color as the remainder of the thallus and did not possess the definite shape or pigmentation of those fully matured. On many of the pieces of shell there adhered numerous red or brown spores which were dislodged with difficulty. Frequently they were also imbedded in depressions which they seem to have dissolved for themselves. In some forms, spores had to be picked out with needles. These multiple spores adhered together in clumps, and were separated with difficulty. These observations would indicate that mature spores are covered with a cementing substance which cause them to adhere to each other and to the surface of other objects.

The molds were isolated from various samples of sand by examining small quantities in Petri dish in xylol. This reagent cleared the specimens promptly and, following the clearing process, they could readily be " picked out " under a binocular microscope. The xylol evaporated completely from the sand and did not alter it in any way. The fragments of shells showing molds were re-

moved from the xylol with a curved, flattened needle and were mounted directly into balsam.

Many pieces of shell were so curved that it was practically impossible to get the entire specimen in the same optical plane. Photography was obviously unsatisfactory in making such records. Where difficulties of this kind were encountered sketches were added to complete the illustrated records of this collection.

The photographs illustrating this article were prepared by Dr. Edwin J. Kohl of Purdue. The photographs were made directly from balsam mounts. The writers wish to acknowledge their indebtedness to Dr. Kohl for his fine contribution to this investigation.

The writers are of the opinion that an unusual habitat for molds has been discovered and that new species may be found by examining calcareous sands from different parts of the world. The better preserved deposits of marl theoretically should offer unusual facilities for conducting these studies.

It is believed that most of the forms that have been examined are Phycomycetous fungi, a few being allied with the Cladochytriaceae. Some may be more closely related to the Myxomycetes.

PURDUE UNIVERSITY,
LAFAYETTE, IND.

EXPLANATION OF FIGURE

Fig. 1. *A*, central sporangium with lateral pouches arranged palmately; *B*, numerous sporangia with ostioles; distributive hyphae originating near the ostiolar openings; *C*, an arborescent species on a shell fragment; *D*, dense population of shell fungi; sporangia and distributive hyphae shown; *E*, thallus, probably a sporangium, with lateral pouches; distributive hyphae arising from the lateral pouches; in shell matrix; *F*, branched thallus form with sporangium opening at the shell surface; note distributive hyphae; *G*, large paramoecium-like form; no lateral pouches; hyphae branch at right angles; note ostiole at the apex of the figure; *H*, simple sporangia united by distributive hyphae; I, spiny shell fungus; *J*, shell fungi in a sponge spicule.

ADDITIONAL DATA ON SEX REACTIONS IN MONOSPORE RACES OF NEUROSPORA TETRASPERMA[1]

BERNICE SEAVER

(WITH 7 FIGURES)

In *Neurospora sitophila,* which is obligately heterothallic, after the fusing nuclei come together in the ascus or spore mother cell, the nuclear spindles are commonly oriented in such a way as to insure the cutting out of eight uninucleate spores. If the factors for sex segregate at the first division, the eight spores in the ascus will be arranged so that the four spores in one end of the ascus will be of one sex and the other four of the opposite sex (Dodge, **1927**). If the factors for sex segregate at the second division, the spores will be arranged in the ascus with two sexes alternating, two spores of one sex and two spores of the other, etc. (Wilcox, **1928**). The spindles, during the second division in *N. sitophila,* lie one directly above the other and longitudinally in the ascus.

In the species *N. tetrasperma,* they more frequently lie at an angle and parallel to each other (Colson, **1934**) but not infrequently (Dodge, **1927**) they appear to lie one above the other, as in *N. sitophila,* although this may sometimes be due to the particular plane in which the ascus was cut in sectioning. This orientation of the spindles in *N. tetrasperma* is such that the spores to be cut out later will have two nuclei instead of one at their origin and, usually these nuclei will be opposite in their sex reactions.

Dodge has assumed that when the spindles lie one above the other in *N. tetrasperma* and segregation of the sex factors occurs at the first division there is something corresponding to a sex attraction strong enough to cause a rearrangement of the four resulting nuclei before the third division takes place. This attrac-

[1] Paper read at a meeting of the Mycological Society of America at Atlantic City, December 30, 1936.

tion would tend to move one nucleus of the upper spindle downward and the one nucleus of the lower spindle upward so that the four nuclei would finally lie two of opposite sex in each end of the ascus, or, in the same position taken by the nuclei following the conjugate type of orientation. In a recent paper Dodge (**1936**) shows how bisexual spores might be cut out without any rearrangement when the sex factors segregate in the second division. He shows also in the same paper how binucleate unisexual spores might be cut out by having the spindles oriented in the same manner, one above the other, but with the sexes segregating at the first division. These are purely hypothetical possibilities. We need further cytological evidence on this point.

Both Dodge and Lindegren have reported finding large unisexual spores. Dodge (**1928**) states, "Single ascospore cultures were made by germinating what were judged to be normal homothallic spores of *Neurospora tetrasperma*. Of some fifty such cultures only one failed to produce perithecia and it was discarded." Lindegren (**1932**), in germinating spores taken from 5-spored asci, found but one ascus out of thirteen which contained three binucleate unisexual spores and two uninucleate spores. Two of the binucleate spores were of one sex and the other binucleate spore and the two uninucleate spores were of the other sex. However, it was assumed that the per cent of these spores was very small, possibly two per cent, but this conclusion was based on very little evidence. At Dr. Dodge's suggestion I undertook to obtain further data.

Ascospores from a 4-spored ascus commonly measure about $31 \times 15\,\mu$. Uninucleate spores from asci containing more than four spores measure about $22 \times 12\,\mu$. My object was to test a larger number of the normal sized spores. The spores were taken from a spore print with a platinum loop and spread on a petri dish of corn meal agar. They were allowed to stand for several hours in order to give the conidia which might have been included accidentally a chance to germinate. Then they were heated for an hour at 55° C. This heating process kills the conidia which have started to germinate and also stimulates the germination of the ascospores. About six hours after they have been heated the spores germinate enough so that they may be cut out

on blocks of agar and removed to tubes of cornmeal agar. As far as possible the normal sized spores were chosen avoiding the small uninucleate ones.

About 1400 spores in all were isolated.[2] Two sets of spores were analyzed. The first were spores from matings of two normal races. The other set were the products of matings of a normal race with a lethal race, the lethal being one that carries a recessive lethal factor, *l*, which when homozygous, or *ll*, in an ascus causes ascus abortion without spore formation. When the ascus is heterozygous, or *Ll*, ascospores are cut out normally. It is impossible to tell the difference by looking at the spores in an ascus, between those from the mother ascus which was homozygous, *LL*, and those from an ascus which was heterozygous, *Ll*, because *L* is dominant and therefore both kinds delimit spores and are otherwise normal in their appearance.

About ten per cent of these normal sized spores (both races included) proved to be unisexual in their reactions. Of spores from the normal, $S_1 \times S_9$, there were 500 isolated and 49 of these were unisexual spores (TABLE 1). Ten of these unisexual spores were of sex *A* and 39 of sex *a*. In the normal plus lethal race there were about 400 spores isolated and 51 of these spores (TABLE 2) were unisexual in their reactions, 30 of sex a and 21 of sex *A*, 22 lethal (homokaryotic) and 29 normal, either homokaryotic *L* and *L* or heterokaryotic *L* and *l*.

TABLE 1

SPORES TESTING AS UNISEXUAL BUT LARGE ENOUGH TO HAVE ORIGINALLY CONTAINED TWO NUCLEI (SPORES FROM MATINGS OF TWO NORMAL STRAINS, $S_1 \times S_9$).

No.	Size	Sex *A*	Sex *a*
3	28 × 15–18 μ	1	2
1	30 × 17 μ	1	
29	31 × 15–18 μ	5	24
2	32 × 15–18 μ	1	1
5	33 × 15–18 μ	1	4
3	34 × 15–18 μ		3
2	35 × 15–18 μ		2
4	37 × 15 μ	1	3
49		10	39

[2] Of these 500 were not considered in the computation because the unisexual spores among this number were not measured. The percentage would not have been changed materially if they had been included.

TABLE 2

SPORES TESTING AS UNISEXUAL BUT LARGE ENOUGH TO HAVE ORIGINALLY CONTAINED TWO NUCLEI (SPORES FROM HETEROZYGOUS ASCI FROM A MATING OF A NORMAL *L* STRAIN, S_9, AND A LETHAL *l* STRAIN, 9.7 C_8).

No.	Size	Lethal		Normal	
		Sex *a*	Sex *A*	Sex *a*	Sex *A*
2	28 × 18 μ			1	1
2	30 × 16–20 μ	1		1	
36	31 × 15–18 μ	10	7	12	7
3	32 × 15–17 μ	2			1
4	33 × 15–18 μ			2	2
2	35 × 17–18 μ		1	1	
2	37 × 15 μ		1		1
51		13	9	17	12

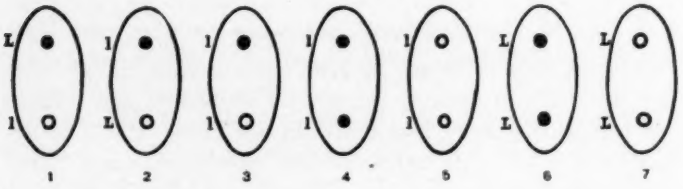
Whether these unisexual spores referred to above actually contain two nuclei of the same sex has not yet been proved. There are two striking facts brought out by the results shown in the tables that would seem to indicate that these spores contain only one nucleus:

First. From some 900 bisexual spores from the mating $S_9 \times 9.7\ C_8$ tested only one spore (FIG. 3) proved to be homokaryotic for the lethal. Of the 49 unisexual spores tested about half of them were homokaryotic for the lethal (FIG. 4, 5) with the sexes about equally represented. That both nuclei should carry the lethal factor is a point not easily understood if there were actually two nuclei in each spore at its origin. That is, binucleate unisexual spores, homokaryotic for the lethal *l* should be no more numerous proportionately than the bisexual spores that are homokaryotic for the lethal. As a matter of fact, however, tests have proved that the former were about 1000 times more numerous. There is no evidence that would indicate a linkage between the lethal and sex factors.

Second. In the progeny from the mating $S_1 \times S_9$ there were many more of the unisexual spores testing as sex *a* than as sex *A*. Theoretically the sexes should be more evenly divided.

It is possible that most of these apparently unisexual spores (FIG. 4, 5, 6, 7) were not originally unisexual but at their origin contained two nuclei which were opposite in their sex reactions

and heterokaryotic for the lethal just as those spores illustrated in figures 1 and 2 but one of the original nuclei was killed or injured, possibly in the process of heating the spore for germination or for some other reason, so that these spores finally contained only active nuclei of one sex. These spores would then test as unisexual and homokaryotic for any other factors. That the division of one of the nuclei may be delayed for a considerable period of time in some cases is likely since occasionally a culture will produce a few perithecia after a much longer period of time than is ordinarily the case with bisexual spores. It is known that when the conidia of such fungi as *Neurospora* and *Aspergillus*



FIGS. 1–7. Diagram of seven possible types of ascospores of *Neurospora tetrasperma* with two nuclei at their origins found in single spore isolations, from matings of a lethal race, and a normal race: *L*, normal factor for spore delimitation; *l*, lethal factor for ascus abortion; black nuclei, sex *A*; white nuclei, sex *a*.

are heated for an hour or two at high temperatures, while they may not be killed, their germination is delayed a long time (Dodge, **1928**). Conidia of *Neurospora* ordinarily would germinate in about 4 hours but when given the heat treatment germination may be retarded as much as 3 days. This probably means that the heat treatment affected the nuclei so that the rapidity with which they divided was slowed up. It may be that the heat treatment necessary for germination sometimes, either kills the nuclei of one sex or greatly retards the rate of division. The S_1 and 9.7 C_8, *a* races, grow more rapidly and seem to be more vigorous than the S_9 and 9.7 C_4, *A* races. It is possible that the *A* nuclei are more susceptible to injury by heating than the a nuclei. This is a difficult point to prove because so few spores of *N. tetrasperma* will germinate without heating.

Having accumulated additional data regarding the normal sized unisexual spores and their sex reactions, the next problem is to determine, if possible, whether or not these really are binucleate at their origin.

In the case of the spores from the normal, $S_1 \times S_9$, mating, this might be proved by germinating spores taken directly from the ascus. If the two sexes were evenly divided among the four spores of an ascus, two of one sex and two of the other, the chances are that the spores were binucleate. If, however, asci could be found in which the sexes were unevenly divided, *i.e.*, three of one sex and one of the other or all four of the same sex we could definitely say that the spores contained living nuclei of only one sex at the time of their germination.

In the case of the spores from the normal plus lethal mating, $S_9 \times 9.8\ C_8$, two methods would have to be used. Sex *a* race produces abundant conidia on potato dextrose agar and since Dodge (**1928**) has proved that in the case of heterokaryotic spores there should be three kinds of nuclei from bisexual mycelia, and two kinds from unisexual mycelia, the proper method here would be to isolate conidia. If the original spore contained two nuclei, homokaryotic as to sex and heterokaryotic as to the lethal factor, by isolating the small conidia we should get condia whose mycelia should eventually produce normal 4-spored asci and also those which would produce aborted asci, when mated. There would be more conidia of the normal type, however, since the lethal factor tends to suppress the conidia somewhat. The *A* sex could not be tested by this method since this sex produces very few conidia, lethal or normal. The best way to analyze these spores would be to cut off hyphal tips and grow them. This should produce the same results as the conidia of sex *a*.

SUMMARY

All normal sized spores of *Neurospora tetrasperma* should contain two nuclei of opposite sex reactions at their origin. Actually only 90 per cent of these proved by experiments to be such. The other 10 per cent tested as unisexual. Three possible explanations for this discrepancy are as follows:

1. If the normal sized spores testing as unisexual contain two living nuclei the sex attraction, previously exerted in the ascus, is not strong enough to *always* bring unlike sexes together in one spore (FIG. 4, 5, 6, 7).

2. If there are nuclei of only one sex after the spore germinates the nuclei of the other sex may have been killed in the process of heating the spore to bring about germination (FIG. 1, 2, 3).

3. If there is only one nucleus that functions it may be that the one nucleus failed to divide when the first nucleus did and was thus crowded out and eventually died, i.e. in the processes (nuclear divisions) leading up to spore delimitations it may be, as Campbell (**1937**) points out for *Gelasinospora tetrasperma,* that one or more of the nuclei failed to divide along with the others and therefore degenerated. This would leave fewer than eight nuclei to be included in the spore complement.

The writer wishes to express her appreciation to Dr. B. O. Dodge, under whose supervision these experiments were conducted.

THE NEW YORK BOTANICAL GARDEN

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EXPLANATION OF FIGURE

Nos. 1–2. Spores of these types, bisexual and heterokaryotic, *L* and *l*, were actually found.

No. 3. One spore of this type, bisexual and homokaryotic, *l* and *l*, was found. There must have been a spore, bisexual and homokaryotic, *L* and *L*, in the same ascus with spore No. 3, but such a spore was not actually found as proved by tests.

Nos. 4–7. Spores large enough to have contained two nuclei at their origin tested as these figures show, unisexual and homokaryotic, *l* and *l*, and *L* and *L*, but whether these spores actually contained two nuclei as the diagram shows has not yet been proved. This could not be proved in the case of Nos. 6 and 7 without introducing a third factor such as a non-conidial factor. Until it is definitely proved that such spores do not contain two nuclei at their origin or do not contain two living nuclei at the time of their germination we will have to assume because of their size that they were as diagrammed.

NOTES AND BRIEF ARTICLES

Three Thousand Mycological Terms

The Rhode Island Botanical Club has sponsored a second publication prepared by the undersigned, entitled "Three Thousand Mycological Terms." It is a glossary of terms used in Mycology, descriptive, taxonomic, morphological and cytological. It is small octavo of 151 pages, and 12 plates of line-drawing illustrations prepared by Henry A. C. Jackson. The price is $2.00 and it may be obtained by forwarding a check to the author.

This glossary has been prepared not for pecuniary profit, but as a small service to English-speaking students of Mycology and Phytopathology. This service can be complete in the future only with the coöperation of other mycologists. Hence, the compiler of this small work will welcome the receipt of new terms and definitions, criticisms of those already included, differences of opinion, and suggestions as to changes of any sort. Only by such coöperation can a future edition be made reasonably complete, up-to-date and adequate.—Walter H. Snell.

The unchanged reprint of Elias Magnus Fries, Hymenomycetes Europaei, which was published in Svea 1874, has now appeared. The price of the book so important for mycological research is R.M. 45,–. It is edited by Dr. Werner Klinkhardt, Leipzig.

The preparation of the Yearbook of the Mycological Society of America will shortly be commenced. Members are urgently requested to send, at their earliest convenience, to the Secretary-Treasurer, 20 Divinity Avenue, Cambridge, Mass., any change in address or other additions or corrections to the subject matter of the Yearbook as it appeared last year.

MYCOLOGIA ENDOWMENT FUND

During the past year a rather strenuous campaign has been conducted to sell the early volumes of MYCOLOGIA. This has given surprising results. From the amount accumulated during the latter part of 1935 and 1936 we have added $2500.00 to our restricted Mycologia Endowment Fund, bringing the total up to $4500.00. This money is invested by the New York Botanical Garden, and interest prorated to MYCOLOGIA each year. It is hoped that this endowment may be increased each year until it is of sufficient size to yield a substantial income to be expended on current issues of MYCOLOGIA.—F. J. SEAVER.

RELIQUIAE FARLOWIANAE

The eighth century of the Reliquiae Farlowianae was issued on February first. The specimens have been selected either because of their rarity, their historic value, or to add to our knowledge of the geographic distribution or host-range. Among the specimens are eight which represent type collections: *Bifusella crepidiformis* Darker; *Aecidium Pereziae* Arth.; *Peridermium guatemalense* Arth. & Kern; *Pileolaria extensa* Arth.; *Puccinia fidelis* Arth.; *Puccinia Tetramerii* Seymour; *Ravenelia Farlowiana* Diet.; and *Ravenelia versatilis* (Pk.) Diet. As exemplified by specimens from the Thaxter Herbarium, the numbering may seem confusing since in a few instances the numbers follow the names of the person who made the determinations. This method of citation has been adopted because Dr. Thaxter followed no definite system of numbering, but rather gave a new series of numbers to each lot of specimens sent out to specialists. The result is that the same number may be found applied to one or more species in unrelated groups, and some of these numbers have appeared in the literature. Therefore, in addition to the arbitrary numbers mentioned, a regular herbarium accession number has been assigned to each specimen in order to avoid future confusion in citation.

In addition the Reliquiae Farlowianae, the Farlow Herbarium will shortly issue the first fifty numbers of the Reliquiae Tuckermanianae, which like the former will be sent out on an exchange

basis. The specimens are those that Edward Tuckerman had obviously intended to issue in continuation of his *Lichenes Americae Septentrionalis*. Among the material are several type collections which it seems highly desirable to distribute as widely as possible. All of the material has been compared with that in the Tuckerman Herbarium in order to insure the identity of the specimens. Also, to illustrate Tuckerman's concept of the taxonomy of the lichens, each specimen is assigned the same name as was applied in the herbarium, this name then being followed by that accepted by Zahlbruckner in his *Catalogus Lichenum Universalis*. When there were two lots of material on different substrata and from widely separated regions as, for example, Massachusetts and South Carolina, or New Hampshire and California, the two specimens are included in the Reliquiae, not only to show in part Tuckerman's concept of the species, but also to give some information as to the geographic variation and range of the species he recognized. While greater duplication would seem permissable because of the historic value of the specimens, most of which are cited in the *Synopsis of North American Lichens,* this has been avoided and duplicate species from different localities or on different substrata have been set aside, with material that is too scanty for distribution in the Reliquiae, for exchange with those institutions which desire a greater representation of the Tuckerman material and which are willing to exchange material of equal historic or taxonomic value.—D. H. LINDER.

A MYCOLOGICAL PILGRIMAGE

Before the last meeting of the American Association for the Advancement of Science at Atlantic City the writer addressed a letter to Dr. H. M. Fitzpatrick, President of the Mycological Society of America, suggesting a trip to the old J. B. Ellis home at Newfield, New Jersey, as a part of our program, if the weather should permit. The weather proved most favorable, and accordingly an impromptu party was made up on Tuesday afternoon, December 29, 1936, Dr. C. L. Shear acting as guide since he had visited this mecca several years earlier.

The distance from Atlantic City was about thirty-five miles and the party, consisting of three car loads, finally succeeded in locating the old home of this famous mycologist. The writer expected to find this place in a condition of dilapidation, but to his surprise the house both externally and internally was in excellent condition, and occupied by Mr. and Mrs. Wasekanez who have owned the place during the last six years. Years ago Professor L. M. Underwood described this place as a little box-like house. The original house while box-like has been extended by numerous additions in the rear, some of which at least must have been there during Ellis' occupancy.

FIG. 1. Front view of the house in which J. B. Ellis lived for the greater part of his life.

It is not surprising that the owner should have regarded this unexpected party with suspicion. These suspicions were soon dispelled, however, when it was explained to him that he was living in a house formerly owned by a famous scientist. After photographs had been taken the lady of the house invited the party in and showed them through the rooms which had formerly housed the Ellis Collection, which later became the nucleus of the mycological herbarium of The New York Botanical Garden.

The writer has in his possession a copy of Ellis' North American Pyrenomycetes, which was purchased directly from Ellis while he

FIG. 2. Side view of the same house showing numerous additions.

FIG. 3. The visiting party. Left to right: H. M. Fitzpatrick, F. J. Seaver, B. O. Dodge, C. L. Shear, G. W. Martin, W. W. Diehl, A. L. Carrion, and C. W. Emmons. This group picture was taken by J. W. Sinden.

was living in this house in Newfield, New Jersey. This copy was purchased in July, 1905, and the letter in Ellis' handwriting is pre-

served in the back of the book. In December of the same year Mr. Ellis died. On at least one other occasion a specimen of a pyrenomycete collected on *Crataegus* while a student in Iowa was sent to Mr. Ellis for determination, the fungus proving to be *Myriangium Duriaei*. This was the extent of the writer's direct communication with Mr. Ellis.

Realizing that all mycologists must have more or less interest in the home and surroundings under which Ellis did his life work, photographs of the house and the visiting party are here reproduced as a matter of historic record. In looking over these sursoundings it is surprising that one could have accomplished so much with such meager facilities, and is a striking illustration of what persistence and energy can do.

The photographs accompanying this note were taken by C. W. Emmons and J. W. Sinden.—F. J. SEAVER.